RESPONSES OF SUBTROPICAL SEAGRASSES TO FLUCTUATIONS IN SALINITY WITHIN AN EXPERIMENTAL FACILITY

By

THOMAS C. CHESNES

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This manuscript is dedicated to Rose Trivigno.
I know that you would be proud.

ACKNOWLEDGMENTS

Words cannot express my appreciation for those who supported me in this endeavor. My supervisory committee has been without a doubt instrumental to my success. I thank Evan Chipouras for his support from the earliest stages of this project, as well as his ability to help me keep things in perspective. I thank Frank Nordlie for introducing me to ecological research and mentoring my undergraduate project. Without him, I certainly would not be in this profession. I thank Stephen Davis, whose enthusiasm for algae is contagious and inspiring. I especially thank Thomas Crisman who helped reintroduce me to my ecological roots. His patience and assistance were essential in getting through a difficult period. Lastly, I thank my chairman Clay Montague, whose mix of humor and scientific curiosity has inspired me to develop a strong scientific ethic, an interest in the workings of the universe (on all temporal and spatial scales), and a never-ending pursuit of truth over accepted ideas.

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RESPONSES OF SUBTROPICAL SEAGRASSES TO FLUCTUATIONS IN SALINITY WITHIN AN EXPERIMENTAL FACILITY

By

Thomas C. Chesnes

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The sparsity of seagrass communities within the ponds and bays of northern Florida Bay may be due to the wide fluctuations of salinity in these habitats. Seven experiments were performed in a facility designed for salinity fluctuation. Three seagrass species, *Thalassia testudinum*, *Halodule wrightii*, and *Ruppia maritima*, were transplanted to the facility and subjected to varying degrees of salinity fluctuation. Treatments were varied according to salinity wave mean, amplitude, frequency, and suddenness (slope) of change. Two experiments crossed the effects of salinity fluctuation with methods of water circulation and reductions in available light. A final experiment, performed in buckets, measured the photosynthetic and internal osmotic responses of seagrasses to fluctuations in salinity.

To assess the loss of photosynthetic material in the seagrasses during the experiments, a green leaf index (GLI) was developed, based on the percent green leaves

present per turion, prorated for partial green coloration. Leaf and rhizome morphometrics were measured prior to and following the treatments.

Thalassia was the most sensitive to salinity fluctuation. Biological parameters were negatively correlated with increasing salinity wave amplitudes, frequencies, and suddenness of change. The effect of salinity fluctuation was dampened when salinity fluctuated within a range of higher salinities. Salinity fluctuation was more of an influence on *Thalassia* survival than the reduction of light.

Halodule condition was impaired by salinity fluctuation, but not to the extent experienced by *Thalassia*. Green leaf indices decreased with increases in salinity wave amplitude, frequency, and slope. The number of turions per sprig correlated negatively with increasing amplitude and more sudden changes in salinity. *Halodule* survival was enhanced by fluctuations within a higher salinity range.

Ruppia was the most resilient of the seagrass species tested. This seagrass was able to survive all of the salinity fluctuation treatments. Increasing the frequency of salinity change did have a negative impact on this seagrass, resulting in lower green leaf indices and number of leaves.

In order to survive fluctuating salinities, seagrasses must regulate their internal osmotic concentrations in relation to their surrounding waters. *Ruppia* osmoregulated more quickly than *Thalassia* and *Halodule* and may be the key to its resiliency.

CHAPTER 1
INTRODUCTION

Since 1881, human activities have disrupted the natural flow of freshwater from the Everglades into Florida Bay (Fourqurean and Robblee 1999). The Everglades watershed has been engineered and managed for agriculture, flood control, and water supply for the growing population in South Florida (Light and Dineen 1994). Water management protocols involving alterations in freshwater flow can change the regime of salinity fluctuations in the downstream estuary. Sudden releases of flood water may create rapid drops in salinity, whereas water held back in times of drought may amplify salinity increases downstream (Montague and Ley 1993). Changes in community structure will be most noticeable in the estuarine salinity fields closest to land (Estevez 2000).

Salinity related problems arise when estuaries receive too much or too little fresh water, or water at improper times (Odum 1970). These habitats are characterized as being harsh due to the salinity changes, especially when compared to the more static conditions typifying marine or freshwater habitats (Deaton and Greenberg 1986). Changes in salinity occur rapidly in the shallow basins located within the northern land margin of Florida Bay. Periods of high freshwater inflow during the onset of the rainy season, in tropical storms (Chesnes 1999), or water management releases can cause areas

with marine strength salinities to become fresh, in some cases within a matter of days (McIvor et al. 1994, Montague and Chipouras 1998).

The meteorologically driven patterns of salinity are clearly seen in the salinity record of Little Madeira Bay, located within the northern land margin of Florida Bay (Figure 1-1). During the course of the dry season, salinity steadily rose from 10‰ (December 1998) to above 30‰ (May 1999). At the onset of the wet season (May/June 1999), salinity dropped nearly 20‰ in the first week, and approximately 30‰ within three.

Acute fluctuations in salinity also occurred during this time period. During July and August of 1998, salinity fluctuated between 1 and 18‰, with a period of approximately 4 days between peaks and troughs. The amplitude of the fluctuation varied over the course of the month (Figure 1-1). Acute fluctuations also occurred in October and December of 1998, January 1999, and again in the late summer/early autumn of 1999. The amplitude of salinity change was higher in July and August of 1999 than in 1998, when changes of over 20‰ were seen over four day periods.

The pattern of salinity change during transition from high to low salinity in the ponds and bays of the land margin of Florida Bay is more acute than during the transition from low to high (Montague and Chipouras 1998). Increases in salinity can be caused by evaporation during the dry season or by winds pushing saline water from Florida Bay into the estuary. The transition to saltier conditions is thought to be more gradual, and the loss of stenohaline freshwater macrophytes may be a more progressive process as the salinity slowly becomes elevated. Conversely, a sharp decline in the abundance of

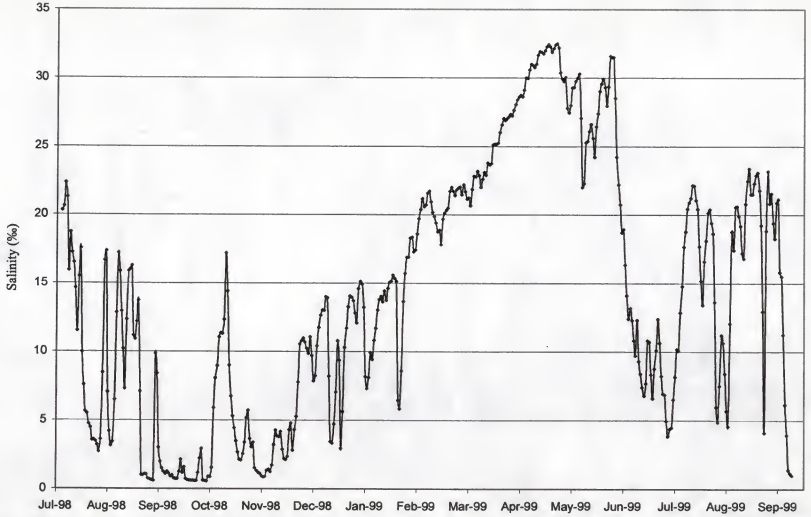


Figure 1-1: Daily average salinity measured at the mouth of Taylor River in Little Madeira Bay. (Data from Patino and Hittle, unpublished)

stenohaline marine macrophytes follows the acute exposure to low salinity at the onset of the rainy season (Montague and Chipouras 1998).

Fluctuations in salinity correlate with decreases in submerged macrophytes and benthic animal density (Montague and Ley 1993, Fears 1993, Jones 1999) as well as fouling organisms (Chesnes 1999). Each individual has a range of salinity tolerance and a narrower range of optimal salinity (Remane and Schlieper 1971). Species richness is especially low in the salinity range between 5 and 8 ‰. This paucity in flora and fauna can be explained by the habitat instability of estuaries (Deaton and Greenberg 1986). The salinity fluctuation hypothesis suggests that this lack of biota may be the result of extreme salinity fluctuation (Montague and Ley 1993). Few organisms have evolved the physiological mechanisms required for life in highly variable environments (Deaton and Greenberg 1986).

Most studies of salinity and submerged macrophytes have focused on tolerances to extreme high and low salinities. Few studies have looked at the effects of salinity fluctuation on submerged aquatic vegetation (Montague and Ley 1993, Fears 1993, Jones 1999, Durako 2000). In some cases, the significance of salinity as a water quality factor is unknown since its effect is confounded with the presence of other environmental conditions (Twilley and Barko 1990). For example, a study by Tomasko and Hall (1999) found that the use of field studies for estimating the lower salinity tolerances of seagrasses might be inappropriate for those systems where water clarity is positively correlated with salinity.

Seagrasses are a vital component of estuarine systems. If salinity fluctuates too much, communities may not become established (Montague and Ley 1993, Montague

1996). Seagrasses provide habitat for many benthic and pelagic organisms (Doering and Chamberlain 2000), stabilize sediments by slowing water movement and increasing sedimentation (Ogden 1980) and can form the basis of the plant-based and detrital-based food chains (Klug 1980). Three seagrass species, *Thalassia testudinum, Halodule* (*Diplanthera*) *wrightii,* and *Ruppia maritima,* are commonly found within the northern land margin of Florida Bay. Little Madeira Bay (Figure 1-1) was included in earlier macrophytes monitoring studies (Montague and Ley 1993, Montague and Chipouras 1998). All three species inhabited these areas during these studies, though *Ruppia* was not as prevalent in the earlier one when salinities were higher and did not fluctuate as much.

The degree to which rapid and dramatic changes in salinity can influence the distribution, abundance, and community composition of seagrasses is unknown. Its importance should be greater at land margins where outwelling freshwater meets saltwater pushed in by coastal tides and winds. If salinity fluctuation is important, then changes in water delivery to the coast that is under control of water managers will be expected to directly influence the distribution and abundance of seagrasses in ways that could affect habitat for fish and birds. Two other physical factors that are affected by features of freshwater discharge have received the most attention by water managers and seagrass scientists, specifically alterations in light and nutrients. In this dissertation attention will be given to a third and perhaps more influential environmental factor, salinity fluctuation.

Thalassia testudinum (turtlegrass) is the dominant marine angiosperm of the Caribbean (Patriquin 1973). It is characterized by a creeping rhizome which has often

been found branched which gives rise to erect branches bearing leaves (Phillips 1960). This species is considered stenohaline (Jagels 1973), although it was found in habitats spanning the entire salinity gradient from fresh to marine strength within the northern land margin of Florida Bay (Montague and Chipouras 1998). Although not thoroughly explored, *Thalassia* may have a temperature optimum near 30° C and a salinity optimum near 30‰ (Zieman 1975). Fluctuations in salinity, especially those ranging into fresher water, may be detrimental to this species.

Halodule wrightii (shoalgrass) had a wider range of salinity tolerance than *Thalassia* in experiments by McMillan and Moseley (1967). In salinity tolerance experiments performed by McMahan (1968), *Halodule* survived in salinities ranging from 9 to 52.2‰, but died in salinities of 3.5‰ and in excess of 70‰. This species was also found in habitats spanning a salinity gradient from nearly fresh to hypersaline within the northern land margin of Florida Bay (Montague and Chipouras 1998). Based on these findings, *Halodule* is expected to be more tolerant to salinity fluctuation than *Thalassia*.

Widgeongrass, *Ruppia maritima,* has a nearly cosmopolitan distribution and worldwide importance as a waterfowl food (Kantrud 1991). *Ruppia* has the widest known range of salinity tolerances of any genus of submerged aquatic vegetation. This species has been found in waters ranging from 0 to 390‰ with optimum growth between 0.5 and 31‰ (Durako 2000). However, a study by McMillan and Moseley (1967) found *Ruppia* to be less tolerant of hypersaline conditions than *Thalassia* and *Halodule*. In the field monitoring studies conducted by Montague and Ley (1993) and Montague and Chipouras (1998) *Ruppia* was found to be common but ephemeral in ponds located along

streams within the land margin of Florida Bay but rarer in the more open, saline bay habitats. Salinity fluctuation may be detrimental to this species despite its reported wide salinity tolerance range.

A facility designed for salinity fluctuation experiments was built in Key Largo, Florida (Anastasiou 1999). Controlled experiments with replicated treatments are possible in this facility. In addition, doing experiments in such a facility gives a researcher the ability to manage, or at least closely monitor, other environmental factors. Within the facility, seagrasses can be subjected to various salinity treatments, which differ in rate, frequency, and amplitude of salinity change. Salinity change and the rate of this change were shown to directly affect the health and growth of transplanted individuals of these species (Jones 1999). In the facility, the responses of the seagrasses can be closely monitored in terms of changes in morphology, productivity, and osmoregulation.

The experimental facility can be used to produce alterations in salinity similar to what was seen in the salinity record during the periods of acute salinity fluctuation (Figure 1-1). In addition, the amplitudes and frequencies of salinity change can be modified to expose seagrasses to more or less extreme conditions. If salinity fluctuation is detrimental to the overall health of seagrasses, reductions in biomass, photosynthetic material, and growth should be evident in the plants exposed to these conditions. The impairment of photosynthetic material should result in reduced primary production. If these resources are required by the plants to maintain the functioning of mechanisms used for osmoregulation, there will be less available for building biomass. Consequently, survival and growth will be impaired. In order to test this hypothesis, seven experiments

were performed to explore the responses of the three seagrasses found at the northern land margin of Florida Bay to varying degrees of salinity fluctuation. An eighth experiment was done in Gainesville, Florida to examine the photosynthesis and salinity acclimation rates of seagrasses subjected to salinity fluctuation.

CHAPTER 2
DESCRIPTION OF FACILITY DESIGNED FOR SALINITY FLUCTUATION AND PILOT STUDY

Description of Facility

A schematic diagram of the facility used for conducting salinity fluctuation experiments is provided in Figure 2-1. The facility is located on the grounds of the National Park Service's Key Largo Ranger Station of Everglades National Park, mile marker 98.6 bayside Overseas Highway (Figure 2-2). A more complete description of the facility and discussion of its design criteria, constraints, construction, and testing is given by Anastasiou (1999). Saltwater for the facility was supplied by a well drilled 13.7 meters deep into porous carbonate rock connected to eastern Florida Bay. Salinity in the well was approximately 36‰. Chlorinated fresh water was supplied by the Florida Keys Aqueduct Authority. The fresh water was held in a 242 m^3 painted concrete reservoir (formerly a swimming pool), which increased residence time for dechlorination.

The seagrass experiments occurred within twelve 1.1 m^3 experimental tanks (Figure 2-3). Salinities were manipulated by manually adjusting valves on a seawater-freshwater mixing manifold (center of Figure 2-3). Up to four different salinity regimes could be delivered simultaneously to randomly chosen replicate experimental tanks by connection of hoses of 3.81 cm diameter to a distribution manifold. In general, water was allowed to flow continuously through each experimental tank at approximately 16 $m^3 d^{-1}$

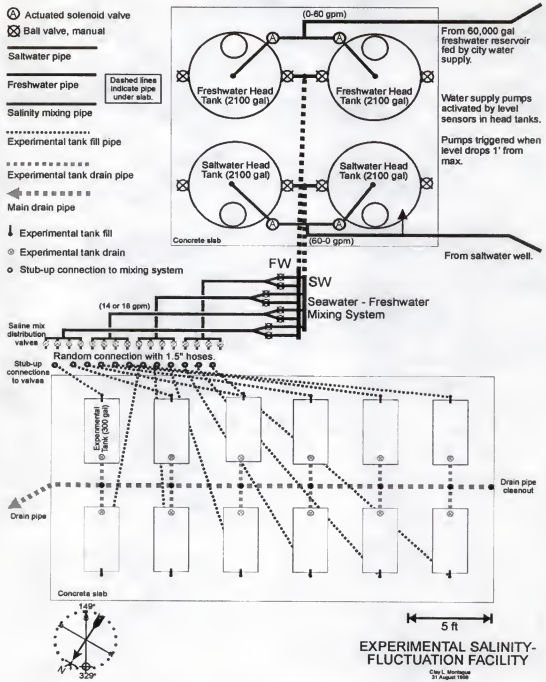


Figure 2-1: Schematic Design of Experimental Facility designed for salinity fluctuation (Anastasiou 1999).

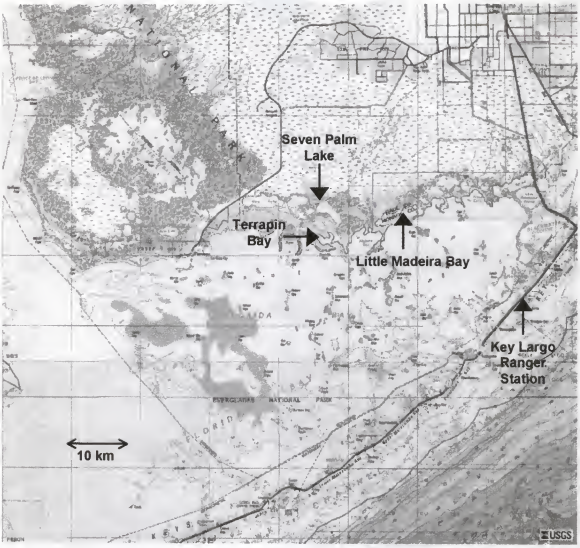


Figure 2-2: Location of collection sites and the Key Largo Ranger Station. (Map from the USGS)

Figure 2-3: Photograph of Experimental Facility. Experimental tanks are in the foreground, storage tanks for fresh and sea water are located on the hill

unless flow was ceased due to experimental design. A constant inflow facilitates water circulation. Water level was maintained at approximately 50 cm by a stand pipe drain.

Salinity was controlled by varying the quantities of fresh and salt water delivered through the mixing manifold to the tanks. When fresh water was delivered to a tank containing water of a higher salinity, an extension was added to the inflow pipe to deliver water to the bottom of the water column. This enhanced mixing and flushing of the higher density water. Complete turnover of a 1.1 m^3 tank from one extreme salinity to another occurred within 2 or 3 hours at an inflow rate of 16 $m^3 d^{-1}$.

Materials and Methods of Pilot Study

Seagrass Collection and Experiment Preparation

Plants used in this experiment were collected in April 1998. Sprigs of *Ruppia* were collected from the northern region of Seven Palm Lake (N 25° 11.69', W 80° 43.42'), and *Halodule* from Terrapin Bay (N 25° 09.49', W 80° 43.84), both areas located in north-central Florida Bay (Figure 2-2). *Thalassia* sprigs were collected from a floating mat found nearby the Key Largo Ranger Station (Figure 2-2). Twenty-four polyethylene tubs (Rubbermaid Inc., measuring 57 X 46 cm) were filled with approximately 22.7 kg of Quikrete Commercial Grade fine sand. *Halodule* and *Ruppia* rhizomes were planted at a density of 40 to 60 percent coverage in separate tubs. Sprigs were selected that had the presence of green leaves and apical meristems. *Thalassia* sprigs were prepared so that each rhizome had only one shoot as well as an apical meristem. In each *Thalassia* tub, five rows of three individual seagrasses were planted, totaling fifteen. Once planted, the tubs of *Ruppia*, *Halodule*, and *Thalassia* were placed in holding tanks of 1.1 m^3 with salinities of 15, 25, and 36‰, respectively. The plants remained at these salinities for approximately two months.

Eight of the facility's twelve experimental tanks were used in this experiment. Salinity fluctuation treatments were assigned randomly to the tanks. Four tanks received a stable salinity treatment (SST1), maintained at 18‰. Two tanks received a four day period of salinity fluctuation (P4D) in which salinity was alternated between 0 and 36‰ every two days. Two other tanks received an eight day period of salinity fluctuation between the same extremes (P8D). One tub of each seagrass was randomly selected from its respective holding tank and placed into a randomly assigned position (North, Center,

or South) within each of the eight experimental tanks. Treatments were applied during the period from June 30 to August 1, 1998.

Protocol of Experiment

Salinity, temperature, and amount of ambient light, light just below the water surface and at the tank bottom were measured daily. The percentage of light reaching the seagrass was computed using Beers Law. Light measurements were taken within two hours of solar noon, in an unshadowed area of each tank, using a quantum photometer (Li-Cor Inc. model LI-185 B). Salinity and temperature were measured using a refractometer (Leica model TS) and mercury thermometer, respectively. Water inflow rates were checked three times a week by timing the filling of a bucket of known volume. Adjustments were made as needed to ensure that each tank was receiving a similar influx of water. Epiphytic algae were removed daily from the seagrass by gently pinching the seagrass blades in an upward motion, and from the tubs and tank walls by scrubbing with a brush. Noticeable turbidity caused by the suspension of epiphytes subsided within an hour of scrubbing.

Daily monitoring of the seagrasses involved counting the number of shoots present, evaluating the color of the leaves, and noting the presence of new leaves. Color was rated using an index (Table 2-1) similar to that used in a related field transplant study (Jones 1999). The presence of green leaves indicated a healthy shoot, while the other colors were assumed to indicate a physiological impairment of the leaf. White leaves were assumed to be dead. An overall estimate of amount of green, yellow, brown, and white coloration was made for *Thalassia* sprigs. Green, brown, and white ratings were made for *Halodule* and *Ruppia* because any yellow stage was too short-lived to be

reliably assessed. A grid was constructed to divide the *Halodule* and *Ruppia* seagrass tubs into nine equal areas of 230 cm^2 each. Color index ratings from Table 2-1 were recorded for each grid region. Color ratings were converted to a percentage, based on the mean percent within the color rating range (Table 2-1).

Complete shoot counts and color analyses of all three species were performed on days 1, 2, 3, 15, and 31 of the experiment. On the other days, two grid regions were randomly selected for coloration surveys and shoot counts in each tub of *Ruppia* and *Halodule*. For *Thalassia* on these interim days, two of the fifteen shoots were randomly selected in each tank for coloration analysis.

Table 2-1: Color rating scale used in pilot study.

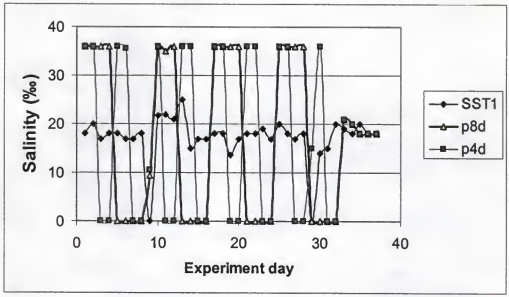
Index Rating	Percent Color Range	Mean %
6	100 % color	100
5	95% to less than 100% color	97.5
4	66% to less than 95% color	80
3	33% to less than 66% color	50
2	5% to less than 33% color	20
1	Greater than 0% but less than 5% color	2.5
0	0% color	0

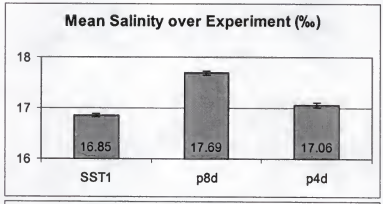
Results of Pilot Study

Physical Parameters

Average daily salinity measurements for each treatment are shown in Figure 2-4. Within the stable salinity tanks, salinity varied from the desired 18‰. Adjustments to the salinity of the treatment tanks caused subsequent changes in the flow rates and head pressure in the other tanks. This adjustment, in turn, caused unintended deviations in the salinity of the tanks designated for stable salinity treatment. Unexpected salinity fluctuations were also caused by mechanical failure. On Days 9 and 29 the saltwater pump failed sometime during the previous night, which resulted in lower salinities than desired within both the experimental and control tanks for an unknown period of time. Nevertheless, the total difference in range of average salinity amongst treatments is less than one part per thousand (Figure 2-4, middle panel) and the standard deviation of salinity was considerably higher in the fluctuating treatments as per the design (Figure 2-4, bottom panel).

Mean temperatures of the treatments were similar, deviating from each other by less than 0.5 ° C (Figure 2-5, top panel). The fraction of surface light reaching seagrass depth exhibited a wider range amongst treatments, however (Figure 2-2, lower panel). Suspended algae that were present primarily in the freshwater storage and delivery system increased the turbidity over that in the seawater system. Seagrasses in the stable salinity treatment (SST1) received the smallest fraction of available light.





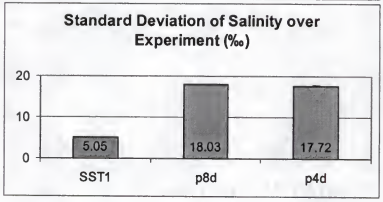


Figure 2-4: Salinity patterns for pilot study. Salinity over time (top panel), mean salinity (middle panel), and standard deviation of salinity (bottom panel) are shown. (Coding is as follows: 1)SST1- Stable salinity treatment, 2) p8d- Square wave with amplitude of 18‰, period of eight days, and 3)p4d- Square wave with amplitude of 18‰, period of eight days)

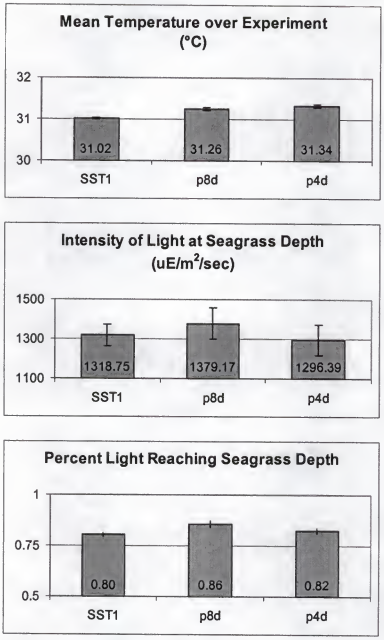


Figure 2-5: Mean temperature (top panel), light intensity at seagrass depth (middle panel), and percent light reaching seagrass depth (bottom panel) for treatments in pilot study. Percent is expressed in decimal form.

Seagrass Measurements

Figure 2-6 shows the average color ratings obtained from *Thalassia*. Ratings are represented by a stacked bar for each experimental day. Green is given at the base of the bar with the other colors sequentially above from chlorotic to white on top. Subsamples varied in coloration for all *Thalassia* treatments (Figure 2-6). It is evident from the initial green coloration ratings that *Thalassia* sprigs used in the SST treatment were in better condition than those in the other treatments (Figure 2-6).

Daily color ratings were transformed to produce accumulations of color ratings over time. At each sample day, color ratings were converted to percent colorations. The percent colorations of each daily measurement were added together and divided by the number of samples. This analysis reduced the noise caused by the subsampling. Color accumulations are given for *Thalassia* in Figure 2-7. Color accumulations are represented by a stacked bar for each experimental day. Green colorations are given at the base of the bar with the other colors sequentially above. *Thalassia* green accumulations increased only in the stable salinity treatment (Figure 2-7, top panel). No green accumulation occurred in the fluctuating treatments over the course of the experiment. Brown and yellow color accumulations decreased with subsequent samples in the fluctuation treatments, replaced with increased white coloration.

Starting green coloration ratings of *Halodule* sprigs were similar (Figure 2-8). Green coloration ratings oscillated around their initial values in the stable salinity treatments, but fell (while white ratings increased) in the fluctuation treatments in samples after ten days of experimentation. Color accumulations were similar over the course of the stable salinity treatment (Figure 2-9, top panel). In both fluctuation

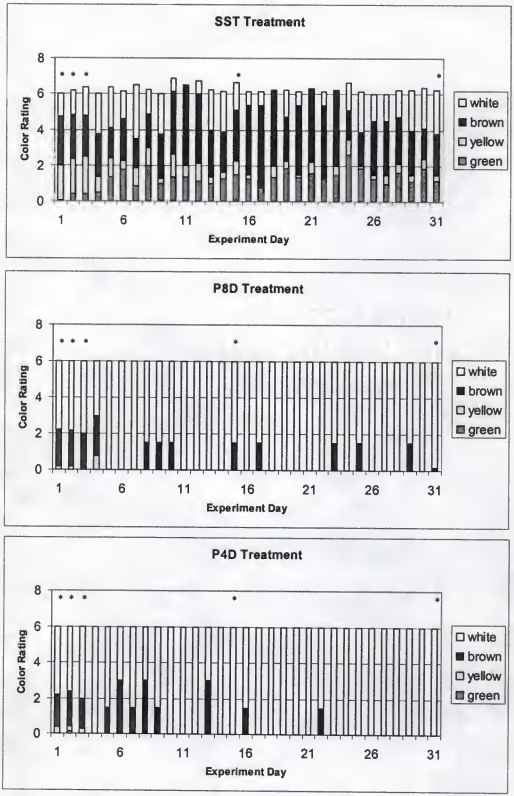


Figure 2-6: Color ratings for *Thalassia* over pilot study. (* denotes ratings derived from average of all plants sampled)

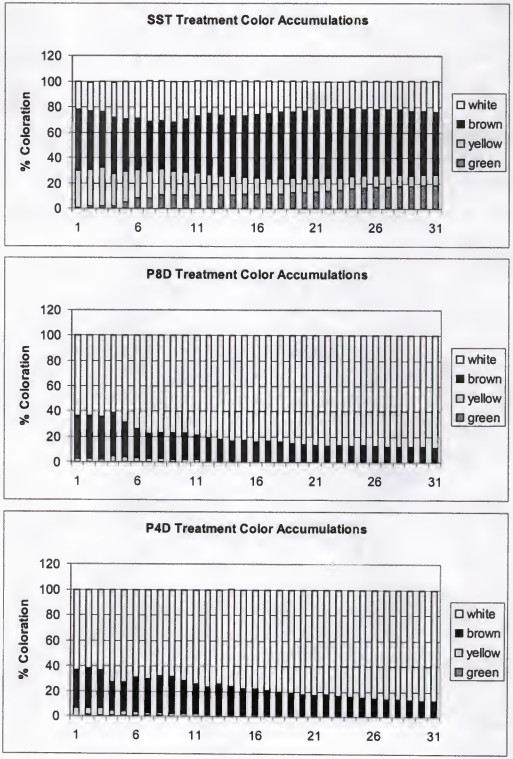


Figure 2-7: Coloration accumulation for *Thalassia* in pilot study.

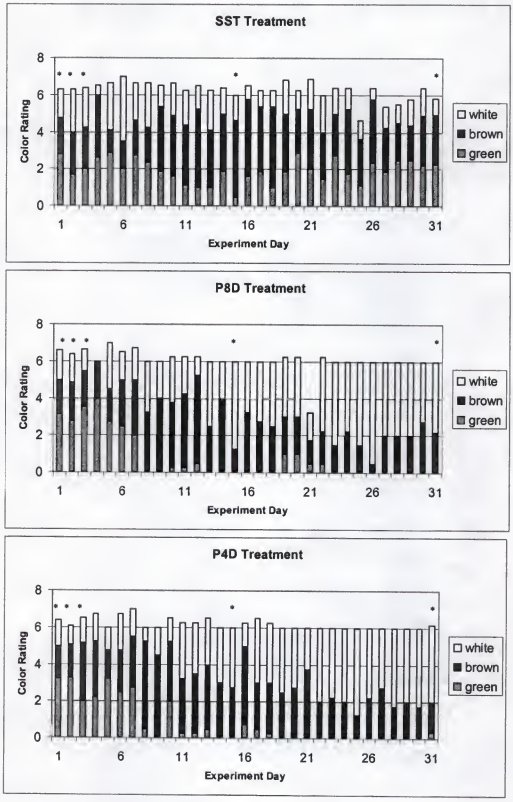


Figure 2-8: Color ratings for *Halodule* over pilot study. (* denotes ratings derived from average of all plants sampled)

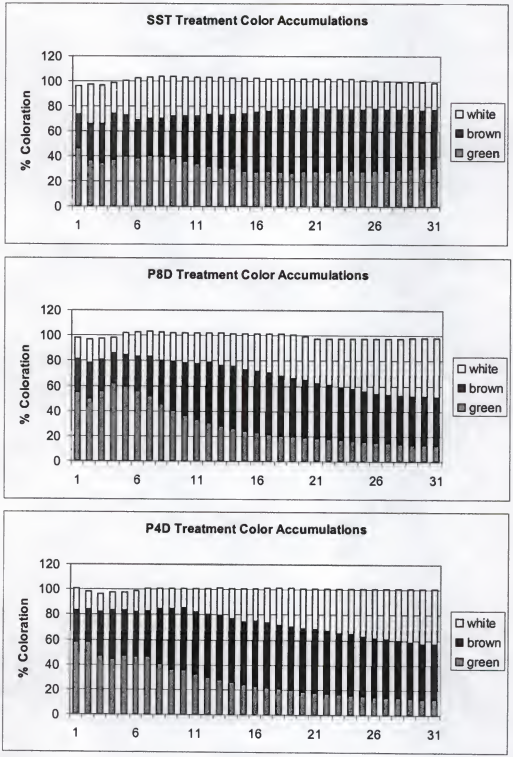


Figure 2-9: Coloration accumulation for *Halodule* in pilot study.

treatments, green color accumulations in *Halodule* decreased, while white accumulations increased.

Green coloration ratings were highest in *Ruppia* exposed to the stable salinity treatment; however; green ratings in the fluctuation treatments persisted throughout the experiment (Figure 2-10). White coloration ratings steadily increased in *Ruppia* over the experiment for all treatments, although greatest white color ratings were seen in the two fluctuation wave treatments. *Ruppia* sprigs exposed to the eight day fluctuation period (P8D) treatment had the greatest decline in green color accumulation (Figure 2-11). Although green color accumulations remained high in the four day fluctuation period (P4D) treatment, white color accumulations increased over the experiment.

Discussion of Pilot Study

The results of the pilot study demonstrate a distinct effect of salinity fluctuation on seagrass survival and growth. Confounding of environmental variables (mean salinity, temperature and light) was minor and was unlikely to have measurably influenced the results. In the three species tested, exposure to fluctuations in salinity resulted in the replacement of healthy green leaf tissue with dead white tissue. Although an effect of fluctuation was clear in all three species when compared with stable salinity, an affect of period of the salinity fluctuation was not evident in the *Thalassia* and *Halodule* experiments. In *Ruppia,* longer periods were more detrimental.

The method of surveillance did not include the monitoring of individual plants. Subsampling did not allow the responses of individual sprigs to be evaluated. Repeated samples on the same plants would be more indicative of the effects over time.

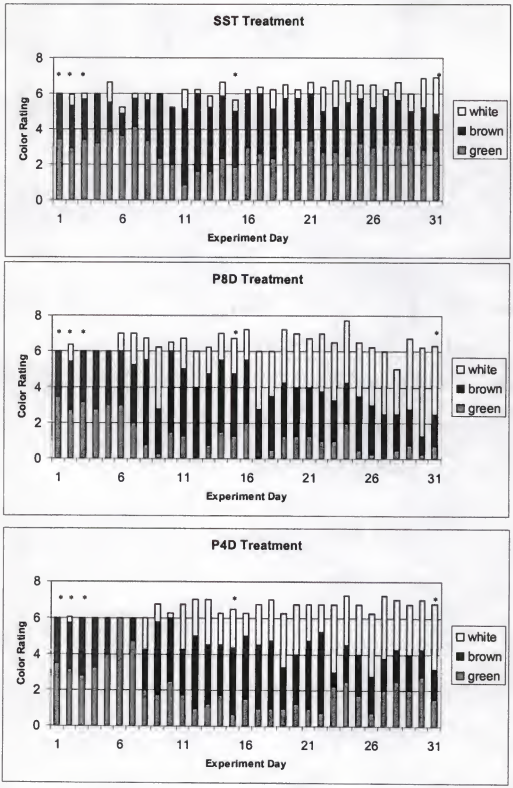


Figure 2-10: Color ratings for *Ruppia* over pilot study. (* denotes ratings derived from average of all plants sampled)

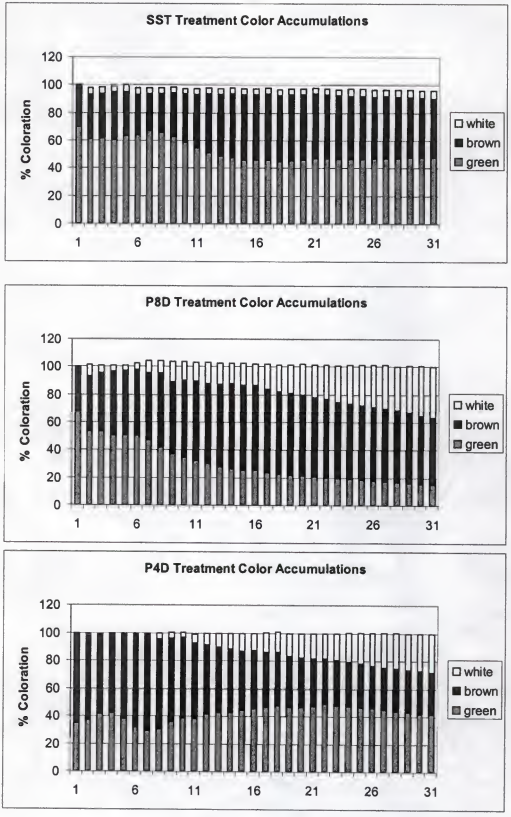


Figure 2-11: Coloration accumulation for *Ruppia* in pilot study.

The effect of salinity fluctuation on *Thalassia* was probably obscured in the pilot study due to the poor starting condition of sprigs planted in the fluctuation treatments. Although trends were evident in the color accumulation analysis, the relatively small amount of starting green coloration may have exacerbated the effects of salinity fluctuation, giving the plants little chance of survival. Acclimation to appropriate salinities and stricter criteria for selection in experiments may remedy this problem in future experiments. Sparse distributions of seagrass communities in northern land margin of Florida Bay may be influenced by the frequent changes of ambient salinity. If seagrasses are losing photosynthetic material due to the affects of salinity fluctuation, energy reserves may not be adequate for vegetative growth and rhizomal elongation, the main methods of reproduction in both *Thalassia* and *Halodule* (McMillan and Mosely 1967, Phillips 1960).

Further experiments are necessary to explore the responses of seagrasses to varying degrees of salinity fluctuation, including the amplitude, frequency, and suddenness of change. In addition, the mean about which salinity fluctuates, light, and nutrient interactions can be examined experimentally. With a more complete and quantitative examination, the distributions and abundances of seagrasses may be predicted using models that include salinity fluctuation as well as light, nutrients, temperature and average salinity.

CHAPTER 3
MATERIALS AND METHODS FOR MAIN STUDY
(EXPERIMENTS 2 THROUGH 7)

Seagrass Collection and Acclimation

All seagrass species used in the salinity fluctuation Experiments 2 through 7 were collected within Little Madeira Bay (N 25° 11.39', W 80° 38.34'), a basin located in the northern land margin of Florida Bay (Figure 2-2). Sprigs of *Thalassia testudinum, Ruppia maritima, and Halodule wrightii* were collected that consisted of a length of rhizome with shoots and roots attached and with healthy leaves. Shoots consist of a sheath (in *Thalassia* and *Halodule)* and at least two leaf blades. Sprigs were carefully removed from the sediment and placed into coolers half filled with water from the collection site. Sprigs of *Halodule wrightii* and *Ruppia maritima* were selected if they had a minimum of three shoots and a growing rhizome tip present. Sprigs of *Thalassia testudinum* had a minimum of two shoots and a growing rhizome tip. Bottom salinity of water sampled from seagrass depth was measured at the collection site with a refractometer. Temperature was measured in the water column with a mercury thermometer attached to a string. In addition, salinity and temperature data from Little Madeira Bay were obtained from the South Florida Information Access (SOFIA) website (Patino and Hittle, unpublished) to determine field conditions in the month prior to collection.

The seagrasses were transported to the experimental facility in coolers and allowed to acclimate in the experimental tanks for a minimum of two weeks. During the acclimation phase, salinities were adjusted from the salinity at the collection site to the mean salinity of the experiments in increments less than 1 ‰ per day. Seagrasses were not monitored during this time. Acclimation periods and ranges for experiments 2 through 7are given in Table 3-1.

Description and Protocol of Facility Experiments

Description of Experiments

The effects of different characteristics of salinity fluctuation were tested in a series of six experiments (numbered 2 through 7). Amplitude, period, suddenness of change and mean salinity were tested. A general description of the experiments is given in Table 3-2, and a more detailed description of treatments is given in Table 3-3. The seven column headings in Table 3-3 describe various aspects of the pattern of salinity fluctuation. The variables tested in each experiment are designated in the shaded column. In all but Experiment 4, fluctuating wave patterns treatments were tested against stable salinity treatments.

In Experiment 3, the effect of the large sudden changes of the square wave was compared to more gradual changes by using a pyramid wave of the same period (8 days) and amplitude (14‰). An example of a pyramid wave and a square wave are shown in Figure 3-1. Frequent salinity changes are required to simulate a pyramid wave in the salinity fluctuation facility. For the pyramid pattern, salinity was changed in a series of small steps of approximately 1.5 ‰ every twelve hours. The effects of constant

Table 3-1: Acclimation periods and ranges for the facility experiments. The end date of the acclimation period was the first day of the experiment.

Experiment	Start Date	End Date	Salinity Range	Duration at Target Salinity (18‰)
2	9/29/98	10/24/98	10 - 18 ‰	10 days
3	12/17/98	1/3/99	12 - 18 ‰	5 days
4	2/6/99	3/3/99	16 - 18 ‰	22 days
5	4/27/99	5/19/99	30 - 18 ‰	5 days
6	6/29/99	7/13/99	18 ‰	15 days
7	10/4/99	10/27/99	7 - 18 ‰	8 days

Table 3-2: Brief Summary of Experiment 2-7. Replicates refer to number of experimental tanks with identical treatments.

Experiment #	Scope of Experiment	Number of Treatments	Number of Replicates Per Treatment
2	Effect of Amplitude	3	4
3	Suddenness of Change (Square vs. Pyramid Wave)	3	4
4	Effect of Mean	2	6
5	Effect of Period	3	3
6	Effect of Water Circulation Method and Salinity Fluctuation (crossed design)	4	3
7	Effect of Light and Amplitude (crossed design)	5	2

Table 3-3: Detailed Summary of Experiments 2-7. Shaded columns designate salinity fluctuation or environmental parameters manipulated in each experiment. Column headings Wave Type, Mean, Amplitude, Period, Salinity Minimum and Maximum describe the salinity fluctuation treatment. Inflow is the rate of which water is delivered to each experimental tank. Percent Sun gives the fraction of sunlight each experimental tank received during an experiment.

Exp 2: Test of the Amplitude of Salinity Fluctuation

	Wave Type	Mean (‰)	Amplitude (‰)	Wave Period (days)	Sal. Min. (‰)	Sal. Max. (‰)	Inflow (liter / sec)	Percent Sun (%)
SST2	stable	18	0	0	18	18	0.13	100
SWa14	square	18	14	4	4	32	0.13	100
SWa7	square	18	7	4	11	25	0.13	100

Exp 3: Test of the Rate of Change of Salinity Fluctuation

	Wave Type	Mean (‰)	Amplitude (‰)	Wave Period (days)	Sal. Min. (‰)	Sal. Max. (‰)	Inflow (liter / sec)	Percent Sun (%)
SST3	stable	18	0	0	18	18	0.13	100
SW	square	18	14	8	4	32	0.13	100
PW	pyramid	18	14	8	4	32	0.13	100

Exp 4: Test of Fluctuation around differing Mean Salinities

	Wave Type	Mean (‰)	Amplitude (‰)	Wave Period (days)	Sal. Min. (‰)	Sal. Max. (‰)	Inflow (liter / sec)	Percent Sun (%)
T9	square	9	9	8	0	18	0.13	100
T27	square	27	9	8	18	36	0.13	100

Exp. 5: Test of Extreme Salinity Fluctuation and Period

	Wave Type	Mean (‰)	Amplitude (‰)	Wave Period (days)	Sal. Min. (‰)	Sal. Max. (‰)	Inflow (liter / sec)	Percent Sun (%)
SST5	stable	18	0	0	18	18	0.13	100
SWp4	square	18	18	4	0	36	0.13	100
SWp8	square	18	18	8	0	36	0.13	100

Exp 6: Water Circulation Method and Salinity Fluctuation

	Wave Type	Mean (‰)	Amplitude (‰)	Wave Period (days)	Sal. Min. (‰)	Sal. Max. (‰)	Inflow (liter / sec)	Percent Sun (%)
SSTt	stable	18	0	0	18	18	0.13	100
SSTb	stable	18	0	0	18	18	0 (air)	100
SWt	square	18	14	8	4	32	0.13	100
SWb	square	18	14	8	4	32	0 (air)	100

Exp 7: Low and High Amplitude Fluctuation Crossed with Full and Reduced Sunlight

	Wave Type	Mean (‰)	Amplitude (‰)	Wave Period (days)	Sal. Min. (‰)	Sal. Max. (‰)	Inflow (liter / sec)	Percent Sun (%)
SSTs	stable	18	0	0	18	18	0 (air)	30
SWa7u	square	18	7	8	11	25	0 (air)	100
SWa7s	square	18	7	8	11	25	0 (air)	30
SWa14u	square	18	14	8	4	32	0 (air)	100
SWa14s	square	18	14	8	4	32	0 (air)	30

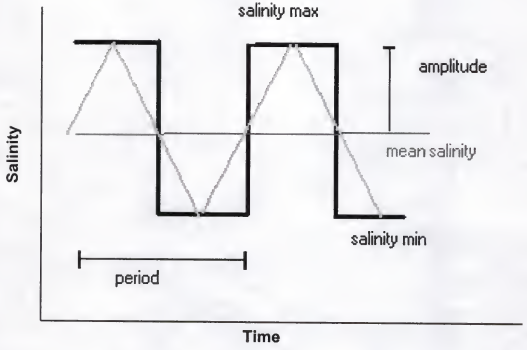


Figure 3-1: Characteristics of a salinity fluctuation wave. The square wave is shown in black, the pyramid wave is gray. Frequency is the reciprocal of period.

throughflow of water versus air bubbling as a means of water circulation in the experimental tanks was tested along with salinity fluctuation in Experiment 6. The effect of shading was tested along with salinity fluctuation in Experiment 7.

In Table 3-3, the column titled "inflow" designates the constant inflow of water into the experimental tanks. For Experiment 6, an air pump was installed to aerate the saltwater head tanks and selected experimental tanks. Aeration helped reduce sulfides in the saltwater head tanks and promoted circulation in the experimental tanks (Anastasiou 1999). Water did not flow through the bubbled experimental tanks, rather they were filled initially with water and aerated. Evaporative losses were replaced daily with freshwater, a task not required in flow through experimental tanks. When it was time to change the salinity, a brief period (approximately 60 minutes) of flow through occurred until the new salinity was achieved.

In Experiment 7, the effects of reduced sunlight were crossed with fluctuation patterns of low and high amplitude. In this crossed design, interactions between light and amplitude can be revealed. This was important because the freshwater at times was more turbid than the saltwater, potentially confounding the results. Randomly selected experimental tanks were covered by 70 % shade cloths to create reduced light conditions (30% full sun). The treatments are designated in Table 3-3 under the column labeled "Percent Sun". *Ruppia* was not tested in this experiment because there was not adequate numbers present at the collection site and other areas of northern Florida Bay where *Ruppia* has been previously found. Furthermore, an unshaded stable salinity treatment was not included because of a lack of acclimated sprigs. An unexpected dieoff of *Thalassia* and *Halodule* occurred during the acclimation period. The stable salinity

treatments of the five prior experiments were unshaded, therefore this treatment was not included to ensure an adequate number of replicates for the remaining treatments tested in this experiment.

Sprig Preparation and Planting

Immediately prior to each experiment, morphometric measurements were taken on all seagrass samples. Sprigs with a growing rhizome tip and at least two shoots with green leaves were randomly selected from the acclimation chamber. The number of shoots was counted. Rhizome length and mature and immature leaf lengths were measured to the nearest half millimeter. Sprigs were then planted into polyethylene tubs (26.5L, Rubbermaid, Inc.), measuring 57 x 46 x 15 cm deep. Fine-grained sand (Quikcrete brand) was used as sediment in all tubs. Two sprigs of each of the three species were planted in each tub in random order, for a total of six sprigs per tub. Three tubs were submerged in each experimental tank, for a total of eighteen sprigs per tank.

Sprig and Tank Monitoring and the Green-Leaf Index

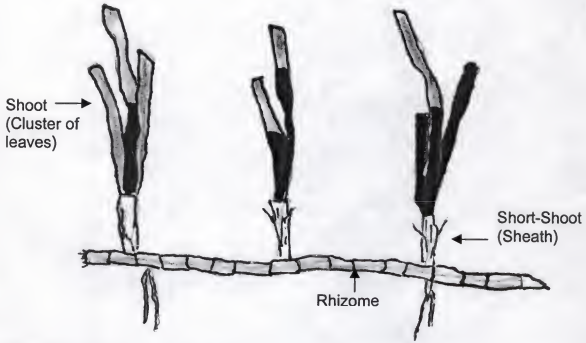
During the course of Experiments 2 through 7, leaves were monitored every two days. The total numbers of leaves were counted on each of the three youngest shoots on the sprig, followed by a count of the number of leaves with any green coloration. Finally, a green-leaf index was assessed for each shoot, based on the number of green leaves present, prorated visually for partial green coloration. For this index, the number of leaves with green coloration on a shoot was multiplied by an estimate of the fraction of all leaves on that shoot that was green. This calculation is performed for the three youngest shoots on a sprig and averaged to give the index value for that sprig. An example of the calculation of the index is illustrated in Figure 3-2.

Daily measurements of salinity, temperature, water inflow, and light were taken in each tank. Epiphytic growths on seagrass leaves were removed daily as in the pilot study. Water samples were collected weekly and sent to the South Florida Water Management District (SFWMD) for analysis of total phosphorus, dissolved inorganic nitrogen, and total Kjeldahl nitrogen. Experiments lasted between 24 and 32 days (Table 3-4). Following an experiment, morphometric measurements were repeated, after which the seagrass sprigs were dried and weighed.

Data and Statistical Analysis

Statistics were performed using statistical software (Statlets Version 1.1B, NWP Associates, Inc.). For each experiment, one-way ANOVA tests were run to compare the responses by each species to each treatment. Fisher's least significant difference (LSD) procedure of was used to identify differences among means of statistically significant ANOVAs. The significance threshold was set at $p < 0.05$.

Pearson product moment correlations and multiple regression analyses were used to explore and quantify relationships between salinity fluctuation and the seagrass responses from among the entire data set of all experiments. For these analyses, salinity fluctuation was quantified across experiments by using seven wave descriptors: mean salinity, standard deviation of salinity, maximum amplitude, suddenness of salinity change, number of salinity changes per day, significant frequency and absolute frequency. The method of calculation for each descriptor is given in Table 3-5. Mean



Percent Green-Leaf (as a decimal):

0 + 0.5 + 0	0.5 + 0.75	1.0 + 0.5 + 1.0

Total per Shoot:

0.5	1.25	2.5

GLI for Sprig (Average of Shoots):

	4.25 / 3	**GLI = 1.42**

Figure 3-2: Example of Green-Leaf Index calculation. For this example, assume black colorations on leaves are green.

Table 3-4: Dates and durations of the six facility experiments.

Experiment #	Treatment Start Date	Treatment End Date	Duration
2	10/25/98	11/25/98	32 days
3	1/4/99	2/3/99	32 days
4	3/4/99	3/29/99	26 days
5	5/20/99	6/13/99	24 days
6	7/6/99	7/31/99	24 days
7	10/28/99	11/22/99	26 days

salinity, standard deviation, and maximum amplitude describe aspects of the amplitude of the salinity waves, where the others address frequency characteristics of the waves. Suddenness gives insight into the rate and magnitude of salinity change by quantifying the maximum slope that occurred in the entire experiment. Due to the exploratory nature of this analysis, the significance threshold was set at $p < 0.001$ to act as a filter to determine the most important correlations.

To account for any confounding effects of salinity fluctuation, correlations involving temperature, light, and water nutrient concentrations were made with plants in the stable salinity treatments only. The influence of these physical parameters will be masked by the detrimental affects of salinity fluctuation, so seagrasses in the fluctuation treatments were excluded from these analyses.

To determine if prior field conditions had any effect on the seagrasses collected, correlations were made between the mean salinities and temperatures measured during the month prior to collection, the salinity at time of sprig collection and the initial green-leaf indices and leaf morphometric measurements of all seagrasses used in the experiments. Rhizome length and shoot number were not analyzed since these measurements are influenced more by the collection process than the field conditions.

Table 3-5: Descriptors to quantify characteristics of salinity fluctuation waves.

Wave Descriptor	Method of Calculation
Mean Salinity	Mean salinity calculated from daily measurements
Standard Deviation of Salinity	Standard deviation calculated from daily measurements
Maximum Amplitude	1/2 (maximum - minimum salinity measurement)
Suddenness of Salinity Change	Maximum slope of salinity wave
Number of Changes Per Day	Number of salinity changes / Number of days
Significant Frequency	1/2 ((number of crossovers -1) / number of days) where "crossover" occurs when salinity crosses designed mean
Absolute Frequency	1/2 (# of peaks + # of troughs / total days)

CHAPTER 4
RESULTS OF EXPERIMENTS 2 THROUGH 7 IN THE SALINITY FLUCTUATION FACILITY ON KEY LARGO

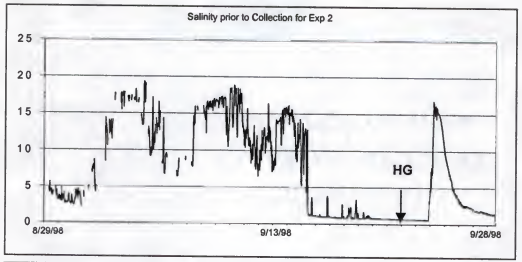
Experiment 2: Effect of Amplitude of Salinity Fluctuation on Seagrass

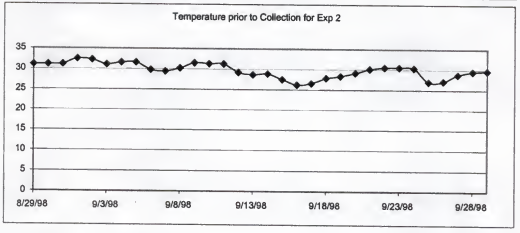
Higher amplitude salinity fluctuation was detrimental to both *Thalassia* and *Halodule* in this experiment. *Ruppia* was more resistant. Biological responses, such as green-leaf index, shoot and leaf number, and rhizome length, were more favorable for *Ruppia* in the lower amplitude fluctuation treatments than in the stable salinity treatment. The response of *Ruppia* in the high amplitude treatment was similar to its response in the stable salinity treatment.

Overview of Physical Measurements

Salinity and temperature measurements during the month prior to plant collection at the mouth of Taylor River in Little Madeira Bay are plotted and summarized in Figure 4-1 (Patino and Hittle, unpublished). The daily temperature averages are calculated from measurements taken at fifteen-minute intervals. Hurricane Georges crossed the collection site on September 23, 1998 (Figure 4-2), during a period of already low salinity. The plants were collected six days after landfall.

The salinity pattern of the three treatments is shown in Figure 4-3. The treatments are coded as follows: 1) SST2- stable salinity treatment, 2) SWa7- square wave treatment with an amplitude of 7‰ and a period of four days, and 3) SWa14- a square wave treatment with an amplitude of 14‰ and a four day period. An indirect hit by Tropical

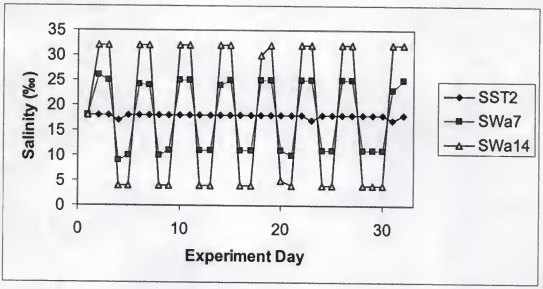


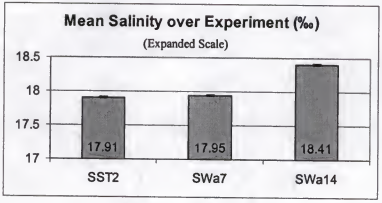


	Salinity (‰)	Temperature (°C)
Mean	7.98	29.5
Standard Deviation	6.74	1.87
Minimum	0.53	25.3
Maximum	19.3	33.56
Measured at Collection	9.5	29.5

Figure 4-1: Salinity (top panel) and temperature (middle panel) in Little Madeira Bay a month prior to seagrass collection (Data from Patino and Hittle, unpublished). Salinity values were measured at 15- minute intervals. Gaps in the salinity record represent where no data were reported. Temperature values are daily averages. Tabular values (bottom panel) are based on data collected at 15-minute intervals (except for data collected at time of collection). Hurricane Georges crossed the collection site on 23 September (designated by HG on the salinity chart).

Figure 4-2: Satellite Image of Hurricane Georges prior to Florida landfall. (Satellite image from National Weather Service)





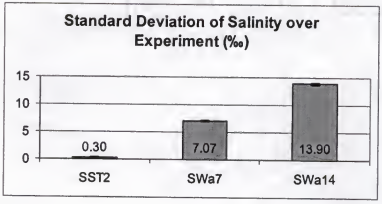


Figure 4-3: Salinity patterns for treatments in Experiment 2. (Coding as follows: 1) SST2- Stable salinity treatment, 2), SWa7- Square wave with amplitude of 14‰, period of 4 days and 3) SWa14- Square wave with amplitude of 14‰, period of 4 days)

Storm Mitch occurred on November 5, 1998, Day 11 of the experiment. The salinity of the treatments was unlikely to have been affected, owing to the 2 to 3 hour turnover time of water through the experimental tanks. Salinity was not measured until the day after landfall. The constant throughflow of water into the experimental tanks may have dampened the affect of rainfall, however. As intended, mean salinities for all treatments were within one-half part per thousand of 18 ‰. The standard deviation of salinity (an index of salinity fluctuation) was proportional to the amplitude of the treatment, as expected (Figure 4-3).

Mean temperatures over the experiment were nearly identical for the three treatments (Figure 4-4, top panel). Average light intensity and the fraction of surface light at seagrass depth were slightly greater in the stable salinity treatment, compared to the low amplitude treatment, but not compared to the high amplitude treatment (Figure 4-4, middle and lower panels).

Mean nutrient concentrations measured over the experiment from the stable salinity treatment outflow and the freshwater and saltwater supplies are given in Figure 4-5. Water from the freshwater source had considerably higher concentrations of TP, TDP, PO4, TKN, and DIN than water from the seawater source. An equal mix of these waters in the 18‰ inflow had intermediate concentrations of the aforementioned nutrients. Nitrite concentrations, however, were highest in the mixed water, with a lesser amount in the freshwater source and practically none in the seawater supply.

Thalassia Measurements

The biological responses of *Thalassia* were negatively influenced by the high amplitude salinity fluctuation treatment. The low amplitude treatment was detrimental as

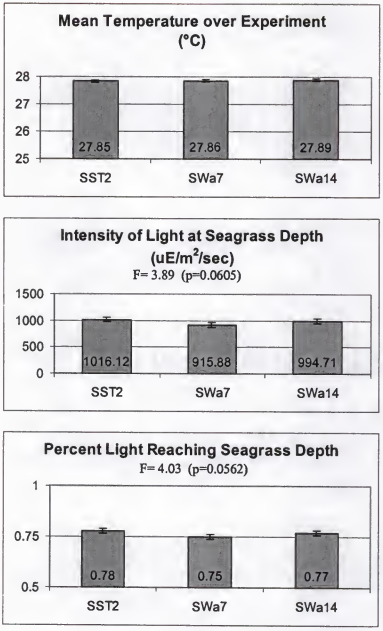


Figure 4-4: Mean temperature (top panel), light intensity (middle panel) and percent light reaching depth of seagrass (lower panel) for treatments in Experiment 2. Error bars are intervals derived from Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time. F ratios are given when differences between means are significant. Percent is expressed in decimal form.

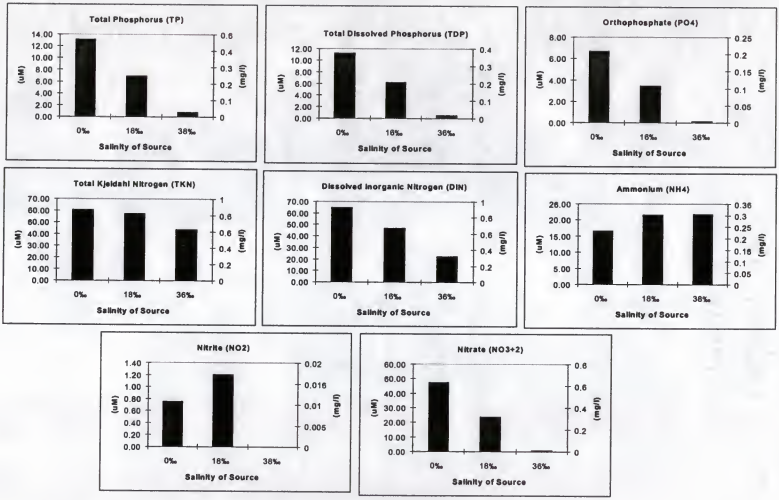


Figure 4-5: Mean nutrient concentrations measured during Experiment 2.

well, but to a lesser extent. Increases in leaf and shoot number were similar between plants in the stable and low amplitude salinity treatments, but were higher than in plants exposed to the high amplitude treatment.

Thalassia green-leaf indices (GLI) declined in all treatments over the first 11 days of the experiment (Figure 4-6, top panel). Plants in the stable salinity treatment however recovered, showing a net increase in GLI over the course of the experiment (Figure 4-6, lower panel). An increase in GLI in plants exposed to the low amplitude treatment occurred during the last seven days of the experiment, but no recovery occurred in plants in the high amplitude treatment.

The number of *Thalassia* shoots had increased by the end of the experiment in all treatments, although this increase was smallest in the high amplitude fluctuation treatment (Figure 4-7, top panel). Rhizome length decreased for plants in all treatments, despite the increase in shoot number (Figure 4-7, middle panel). Belowground biomass in *Thalassia* was greatest in plants exposed to the high amplitude treatment, although not statistically different from plants in the other treatments (Figure 4-7, bottom panel).

For all treatments, *Thalassia* leaves were shorter after the experiment than they were initially, however plants in either the stable salinity or the low amplitude treatments had over 30% more leaves on average after the experiment (Figure 4-8). Those that were in the high amplitude treatment had only 46% of original measurements. Sprigs of *Thalassia* exposed to the higher amplitude salinity fluctuation averaged less than one leaf per shoot (0.63), whereas those in the low amplitude and stable salinity treatments averaged 1.71 and 1.58 leaves per shoot, respectively (Figure 4-8). No statistically significant difference was observed among aboveground biomass. Average shoot,

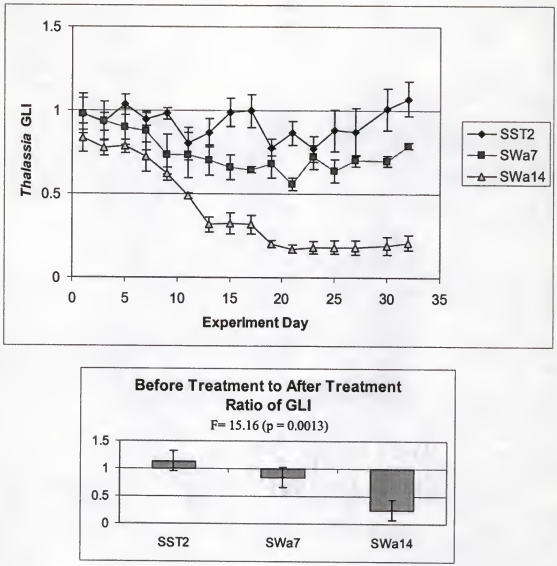


Figure 4-6: Green-leaf indices for *Thalassia* over Experiment 2 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on ratio chart are intervals derived from Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

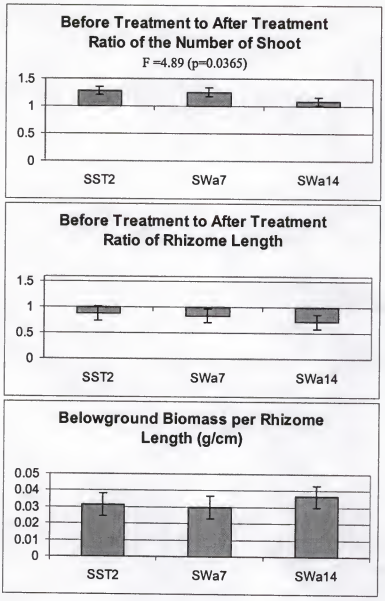


Figure 4-7: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and belowground biomass (bottom panel) after treatments for *Thalassia* sprigs in Experiment 2. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

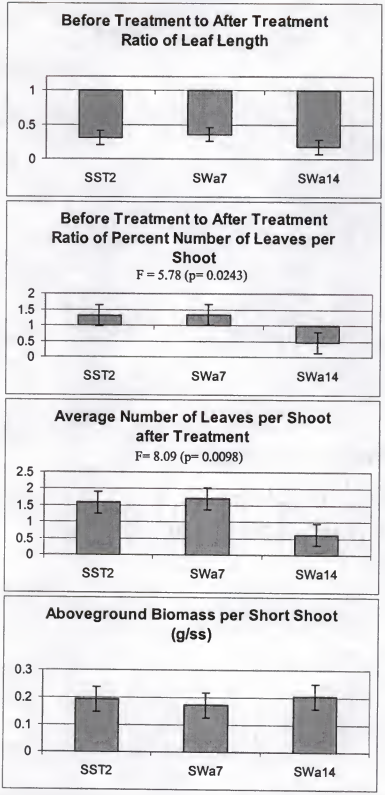


Figure 4-8: Leaf characteristics for *Thalassia* after Experiment 2. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

rhizome, and leaf measurements, and the standard deviations of the measurements, are given in Table 4-1.

Halodule Measurements

Halodule exposed to the high amplitude salinity fluctuation treatment had lower green-leaf indices and a greater reduction in leaf length and number than plants exposed to the other two treatments. *Halodule* tolerated the low amplitude treatment, having similar responses in green-leaf index and leaf length as plants in the stable salinity treatment.

Halodule green-leaf index declined overall under all three treatments, however (Figure 4-9). An increase in GLI for plants in all treatments occurred during the final seven days of the experiment, although the increase was most rapid in the stable salinity treatment. Those in the low amplitude treatment fared the best, although not statistically significantly better than those in the stable salinity treatment according to the ANOVA. As with *Thalassia*, the high amplitude treatment resulted in the greatest decline in *Halodule* green-leaf index (Figure 4-9, bottom panel).

The number of *Halodule* shoots increased most in the low amplitude (SWa7) treatment and least in the stable salinity treatment (Figure 4-10, top panel). The increase in rhizome length in the high amplitude treatment, however, was not statistically different from that in the two other treatments (Figure 4-10, middle panel). *Halodule* sprig biomass was similar among treatments (Figure 4-10, bottom panel).

Average leaf length increased in *Halodule* sprigs under stable salinity and low amplitude treatments. A decrease was observed in the high amplitude treatment (Figure

Table 4-1: Averages and standard deviations of morphometrics measured on *Thalassia* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SST2	2.92	0.83	3.67	1.27
SWa7	3.00	0.85	3.70	1.15
SWa14	3.08	0.83	3.38	1.44
Rhizome Length (cm)				
SST2	22.73	11.97	19.75	10.68
SWa7	22.16	9.29	19.37	7.82
SWa14	24.64	10.79	17.15	8.93
Leaf length (cm)				
SST2	9.78	2.92	2.58	2.00
SWa7	8.73	3.79	2.81	1.77
SWa14	8.47	4.03	1.24	1.48
Leaf Number				
SST2	1.38	0.52	1.58	1.06
SWa7	1.30	0.45	1.74	0.70
SWa14	1.32	0.46	0.63	0.65

4-11, top panel). Those in the low amplitude treatment did not change (Figure 4-11, middle panel). The average number of leaves per shoot in *Halodule* plants under stable salinity were similar to those in high amplitude treatments. The low amplitude treatment had the greatest number of leaves, averaging two leaves per shoot (Figure 4-11, bottom panel). Average shoot, rhizome, and leaf measurements, and the standard deviations of the measurements, are given in Table 4-2.

Ruppia Measurements

In contrast to *Thalassia* and *Halodule, Ruppia* fared worst in the stable salinity treatment. Increases in shoot number and leaves per shoot occurred in plants exposed to both the low and high amplitude treatments, whereas decreases in these measurements occurred in sprigs in the stable salinity treatment.

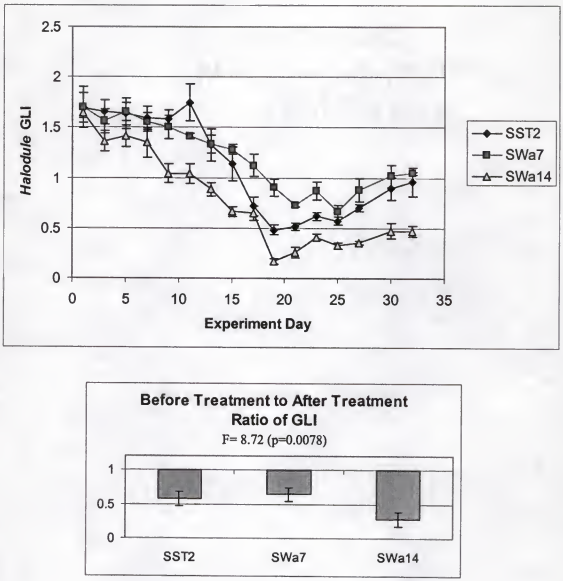


Figure 4-9: Green-leaf indices for *Halodule* over Experiment 2 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time. F ratios are given when differences between means are significant.

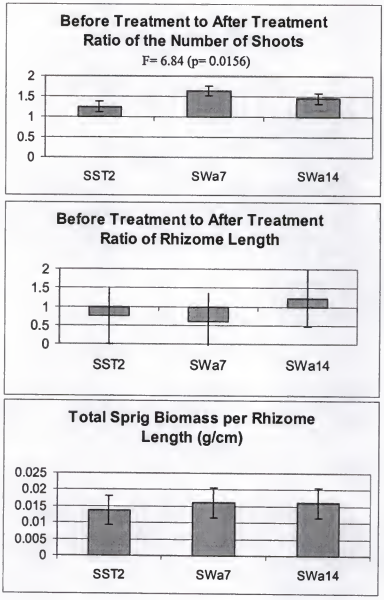


Figure 4-10: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and total sprig biomass (bottom panel) for *Halodule* in Experiment 2. Intervals around means are derived from Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

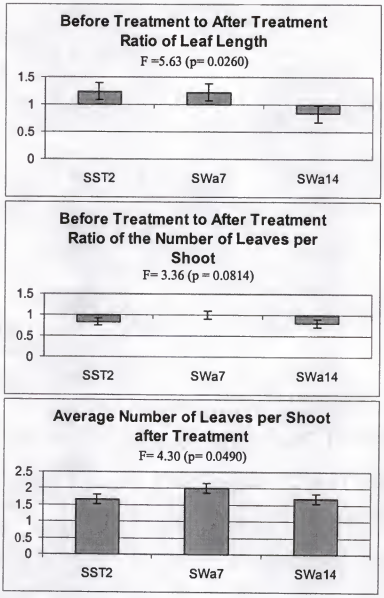


Figure 4-11: Leaf characteristics for *Halodule* after Experiment 2. All percents are expressed in decimal form. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

The greatest decline in green-leaf index for *Ruppia* occurred in the stable salinity treatment (Figure 4-12, bottom panel). Declines were seen in all treatments during the middle period of the experiment, but these were far more dramatic in the stable and high amplitude treatments (Figure 4-12, top panel).

Ruppia sprigs exposed to both the low and high amplitude treatment had more shoots at the conclusion of the experiment, whereas those in the stable salinity treatment had less (Figure 4-13, top panel). *Ruppia* rhizome length nearly tripled (from 2.6 to 9.9 cm) in the low amplitude treatment (Figure 4-13, middle panel), but no significant differences were observed in total sprig biomass among treatments (Figure 4-13, bottom panel).

Table 4-2: Averages and standard deviations of morphometrics measured on *Halodule* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SST2	7.39	3.20	8.70	3.18
SWa7	6.58	2.47	10.13	3.53
SWa14	5.38	2.41	7.38	2.75
Rhizome Length (cm)				
SST2	17.70	7.96	15.36	12.71
SWa7	18.18	9.87	13.00	9.80
SWa14	16.99	9.43	10.79	8.44
Leaf length (cm)				
SST2	5.36	1.40	6.30	2.22
SWa7	5.86	2.02	6.12	1.81
SWa14	5.42	1.57	4.40	1.66
Leaf Number				
SST2	2.07	0.58	1.65	0.43
SWa7	2.17	0.70	2.00	0.68
SWa14	2.19	0.56	1.69	0.54

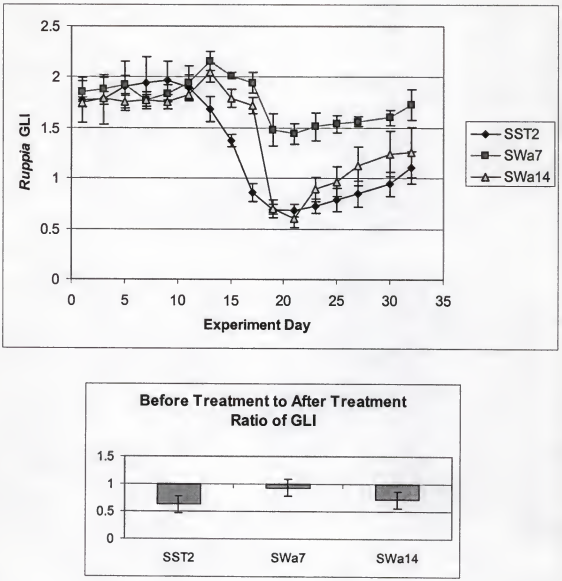


Figure 4-12: Green-leaf indices (GLI) for *Ruppia* over Experiment 2 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time. F ratios are given when differences between means are significant.

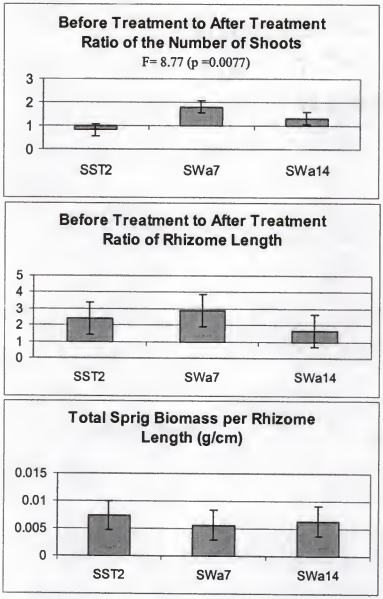


Figure 4-13: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and total sprig biomass (bottom panel) for *Ruppia* in Experiment 2. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Leaf length declined in *Ruppia* plants in the stable salinity and high amplitude treatments (Figure 4-14, top panel). A slight increase (1%) occurred in the low amplitude treatments. The number of *Ruppia* leaves increased in both the low and high amplitude treatments, whereas a slight decrease occurred in the stable salinity treatment (Figure 4-14, middle panel). This treatment also had the least numbers of leaves after the experiment, however no statistically significant differences were observed between the treatments (Figure 4-14, bottom panel). Average shoot, rhizome, and leaf measurements, and the standard deviations of the measurements, are given in Table 4-3.

Experiment 3: Rate of Change of Salinity Fluctuation (Square vs. Pyramid Wave)

Salinity fluctuation was detrimental to *Thalassia*, regardless of the rate of change of salinity. *Halodule* was in poor condition after all treatments, although the decline in green-leaf index was slightly less in the stable salinity treatment than in the fluctuation treatments. *Ruppia* was most negatively affected in the pyramid wave (gradual change) treatments relative the stable salinity and square wave treatments, but not to the extent experienced by the other two seagrasses.

Overview of Physical Measurements

Salinity at the collection site ranged from nearly fresh to about 13‰ during the month prior to seagrass collection, with a mean around 5‰ (Figure 4-15). Mean temperature was approximately 25° C.

The salinity pattern of the three treatments is shown in Figure 4-16. The treatments are coded as follows: 1) SST3- stable salinity treatment, 2) SW- square wave with an amplitude of 14‰ (a range from 4 to 32‰), and a period of eight days (a salinity

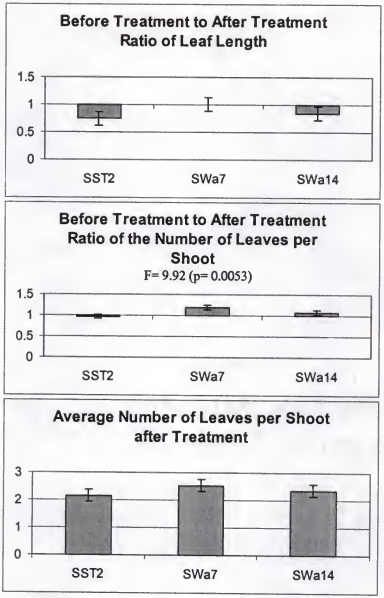


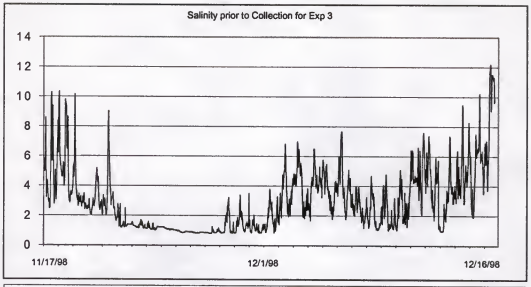
Figure 4-14: Leaf characteristics for *Ruppia* after Experiment 2. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

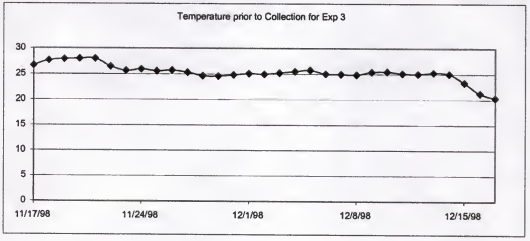
Table 4-3: Averages and standard deviations of morphometrics measured on *Ruppia* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SST2	5.67	1.98	4.38	2.29
SWa7	6.13	1.57	11.04	4.81
SWa14	7.00	2.83	9.48	5.96
Rhizome Length (cm)				
SST2	2.06	1.66	3.54	5.21
SWa7	2.60	1.94	9.89	7.05
SWa14	3.18	3.03	5.69	4.87
Leaf length (cm)				
SST2	5.75	1.43	4.08	1.37
SWa7	5.88	1.27	5.73	1.47
SWa14	5.64	1.62	4.44	0.68
Leaf Number				
SST2	2.37	0.55	2.17	0.60
SWa7	2.31	0.62	2.54	0.50
SWa14	2.32	0.67	2.36	0.54

32‰, adjusted twice daily in 1.5‰ increments. Although the designed mean salinity for all treatments was 18‰, the actual means ranged between 18 and 19‰ (Figure 4-16, middle panel). Major deviations from the designed salinity pattern occured on Days 8 and 14, due to mechanical problems involving the freshwater pump.

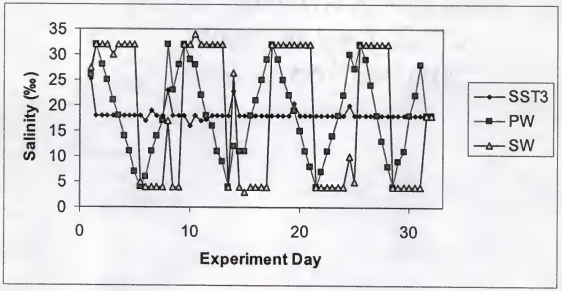
Mean temperatures among treatments differed less than 0.2 °C (Figure 4-17, top panel). Light intensities at seagrass depth as well as water clarity (as indicated by percent light at bottom) are statistically similar amongst treatments (Figure 4-17, middle and bottom panels). Total phosphorus concentrations were an order of magnitude higher in the freshwater source than in the seawater source, whereas total Kjeldahl nitrogen and ammonium concentrations were fairly similar (Figure 4-18).

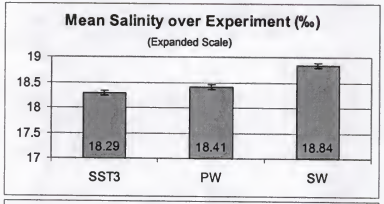




	Salinity (‰)	Temperature (°C)
Mean	4.8	25.3
Standard Deviation	3.12	1.73
Minimum	0.79	18.92
Maximum	13.2	29.6
Measured at Collection	12	21

Figure 4-15: Salinity (top panel) and temperature (middle panel) in Little Madeira Bay a month prior to seagrass collection (Data from Patino and Hittle, unpublished). Salinity values were measured at 15- minute intervals. Temperature values are daily averages. Tabular values (bottom panel) are based on data collected at 15-minute intervals (except for data collected at time of collection).





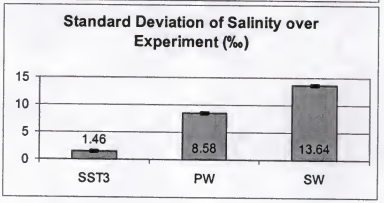


Figure 4-16: Salinity patterns for treatments in Experiment 3. (Coding as follows: 1) SST3- Stable salinity treatment, 2) PW- Pyramid wave with amplitude of 14‰, salinity changing every 12 hours by 1.5‰, and 3) SW- Square wave with amplitude of 14‰, period of 8 days)

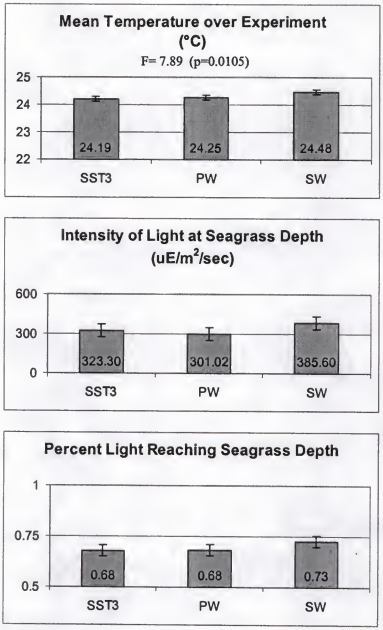


Figure 4-17: Mean temperature (top panel), light intensity (middle panel) and percent light reaching depth of seagrass (bottom panel) for treatments in Experiment 3. Error bars are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time. F ratios are given when differences between means are significant. Percent is expressed in decimal form.

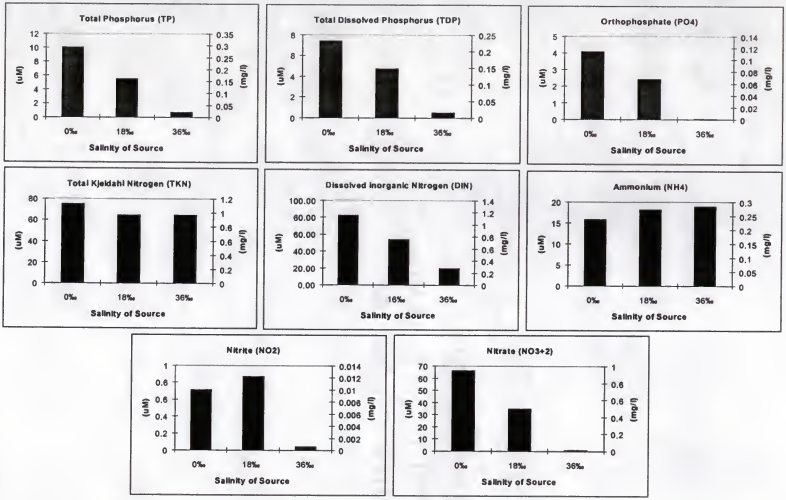


Figure 4-18: Mean nutrient concentrations measured during Experiment 3.

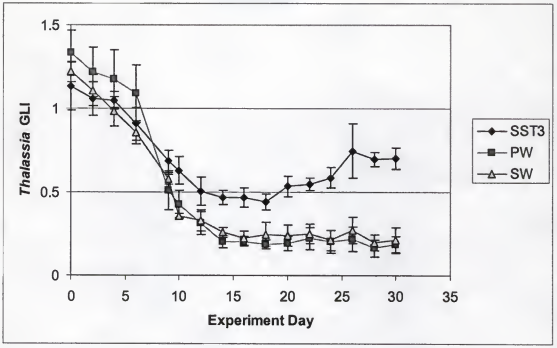
Thalassia Measurements

Thalassia was affected by salinity fluctuation, regardless of the rate of change. Continual, gradual changes were more detrimental to *Thalassia* than sudden changes. In this treatment, the greatest declines of green-leaf index, rhizome length, leaf length and number occurred.

Green-leaf indices (GLI) for *Thalassia* declined in all treatments over the course of this experiment, however, an increase in GLI occurred in the stable salinity treatment during the final third of the experiment (Figure 4-19). The overall reduction in green-leaf index in the stable salinity treatment was less than in the other treatments.

The number of shoots did not change significantly in any treatment (Figure 4-20, top panel). Decreases in rhizome length occurred in all treatments, however. The greatest occurred in the pyramid wave treatment (Figure 4-20, middle panel). *Thalassia* rhizomes from the stable salinity treatment retained the most biomass, significantly moreso than in the square wave treatment (Figure 4-20, bottom panel).

Average leaf length declined during the experiment in all treatments (Figure 4-21, top panel). The number of *Thalassia* leaves per shoot decreased least in the stable salinity treatment (Figure 4-21, second panel). Plants in the pyramid wave treatment ended the experiment with 20% fewer leaves than initially present. *Thalassia* averaged 0.35 leaves per shoot (approximately one leaf per three shoots) after the pyramid wave treatment, in contrast to the square wave (0.76) and stable salinity (1.01) treatments (Figure 4-21, third panel). Aboveground biomass was greatest in the stable salinity treatment. The difference in aboveground biomass between the two fluctuation treatments



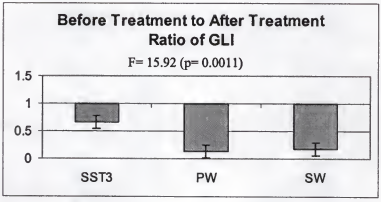


Figure 4-19: Green-leaf indices for *Thalassia* over Experiment 3 (top panel) before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals derived from Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

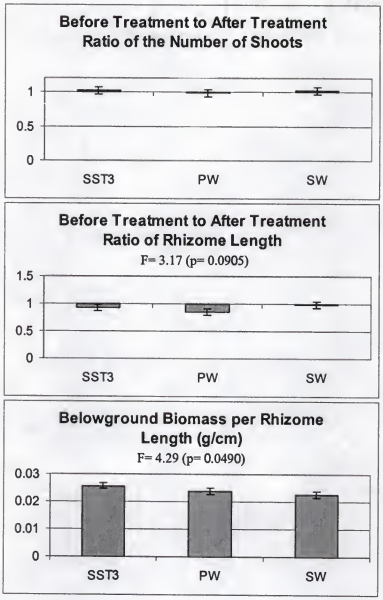


Figure 4-20: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and belowground biomass (bottom panel) for *Thalassia* in Experiment 3. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

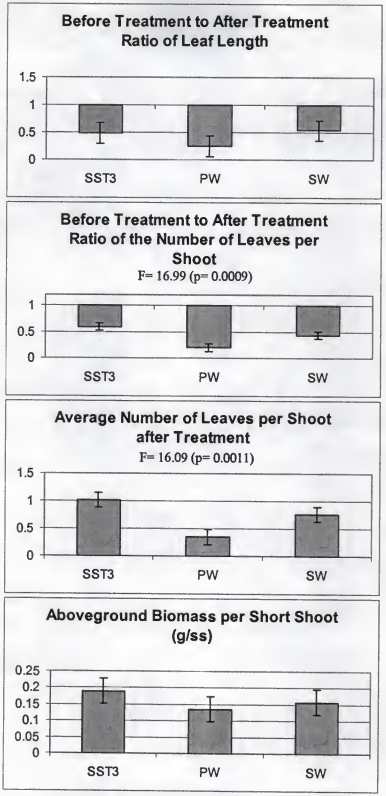


Figure 4-21: Leaf characteristics for *Thalassia* after Experiment 3. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Table 4-4: Averages and standard deviations of morphometrics measured on *Thalassia* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SST3	3.63	0.92	3.71	1.16
PW	3.46	0.93	3.33	0.82
SW	3.96	1.46	3.96	1.63
Rhizome Length (cm)				
SST3	28.25	6.22	26.52	7.01
PW	28.29	9.03	24.83	10.79
SW	30.29	10.15	30.63	13.38
Leaf length (cm)				
SST3	6.07	3.24	2.55	1.98
PW	5.82	2.21	1.44	2.32
SW	6.67	2.49	3.49	3.20
Leaf Number				
SST3	1.69	0.66	1.01	0.90
PW	1.67	0.45	0.35	0.52
SW	1.74	0.54	0.76	0.59

was not statistically significant. Average shoot, rhizome, and leaf measurements, and the standard deviations of the measurements, are given in Table 4-4.

Halodule Measurements

Halodule fared poorly in all treatments, with decreases in green-leaf index, leaf number and length occurring in each. Surprisingly, shoot numbers increased more in the fluctuation treatments.

A sharp decline in GLI occurred in *Halodule* by the ninth day of the experiment in all treatments (Figure 4-22). In the stable salinity treatment, green-leaf indices increased during the remainder of the experiment. Overall, the percent changes of GLI did not differ significantly amongst treatments.

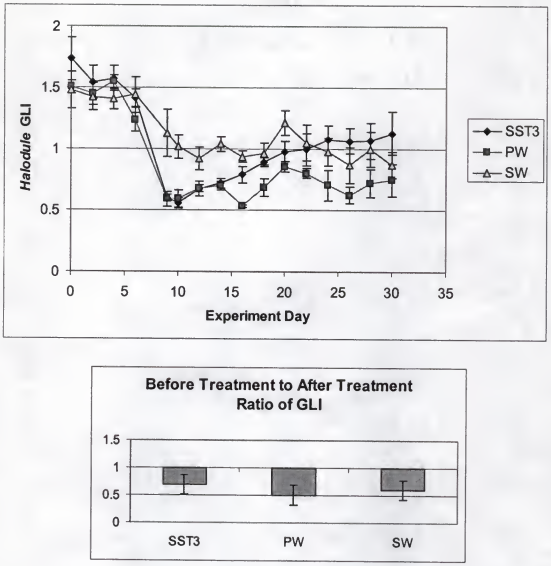


Figure 4-22: Green-leaf indices for *Halodule* over Experiment 3 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

The number of *Halodule* shoots in both the square wave and pyramid wave treatments increased more than in the stable salinity treatment (Figure 4-23, top panel). A slight increase in rhizome length occurred in the square wave treatment; decreases were seen in the others (Figure 4-23, middle panel). The higher *Halodule* biomass in the stable salinity treatment was not statistically different from the other treatments (Figure 4-23, bottom panel).

The decline in average leaf length and number of leaves per shoot was similar in all treatments (Figure 4-24, top and middle panels). In addition, the average numbers of leaves per shoot after the experiment were very close, with 1.47 leaves per shoot in stable salinity treatment versus 1.42 leaves counted in both the square and pyramid wave treatments (Figure 4-24, bottom panel). Average shoot, rhizome, and leaf measurements, and the standard deviations of the measurements, are given in Table 4-5.

Ruppia Measurements

As for *Halodule*, *Ruppia* green-leaf index declined in all treatments during the first third of the experiment, but increased afterwards in the stable salinity treatment (Figure 4-25). Although those in the pyramid wave treatment declined most in GLI, differences among the treatments were statistically insignificant. Declines in *Ruppia* shoot number, rhizome length, leaf length, and leaf number were greatest in the pyramid wave treatments, although statistically significant differences occurred only in rhizome length (top and middle panels of Figure 4-26 and 4-27). Average shoot, rhizome, and leaf measurements, and the standard deviations of the measurements, are in Table 4-6.

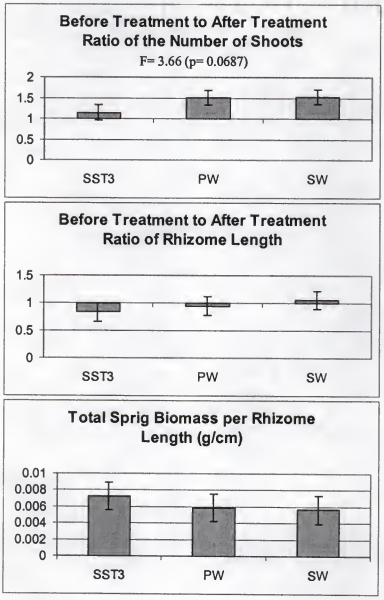


Figure 4-23: Before to after treatment ratios of shoot number (top panel), and rhizome length (middle panel), and total sprig biomass (bottom panel) for *Halodule* in Experiment 3. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

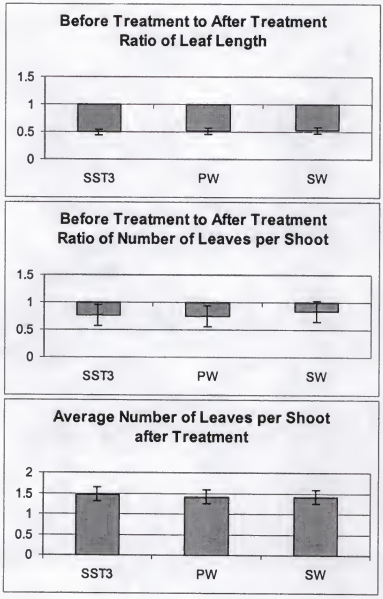
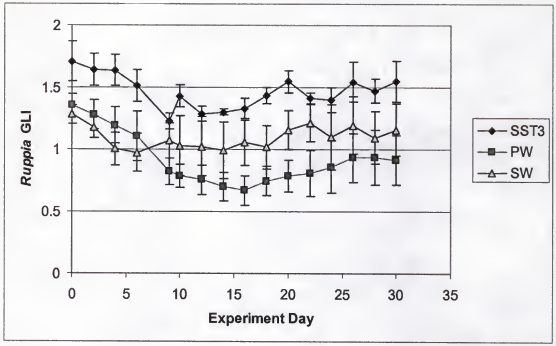


Figure 4-24: Leaf characteristics for *Halodule* after Experiment 3. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Table 4-5: Averages and standard deviations of morphometrics measured on *Halodule* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SST3	5.29	2.18	5.87	2.67
PW	4.71	2.29	6.88	2.85
SW	5.00	1.84	7.17	3.06
Rhizome Length (cm)				
SST3	9.42	4.02	7.70	4.19
PW	10.29	2.88	9.90	6.15
SW	8.96	4.33	8.67	3.68
Leaf length (cm)				
SST3	5.89	1.72	2.68	1.20
PW	6.18	1.74	2.81	1.12
SW	5.73	1.91	2.84	0.75
Leaf Number				
SST3	2.11	0.52	1.42	0.65
PW	2.04	0.53	1.42	0.58
SW	1.96	0.60	1.42	0.36



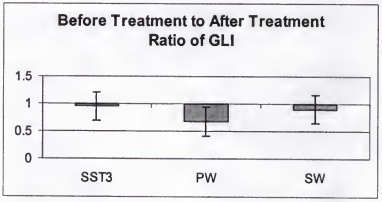


Figure 4-25: Green-leaf indices for *Ruppia* over Experiment 3 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

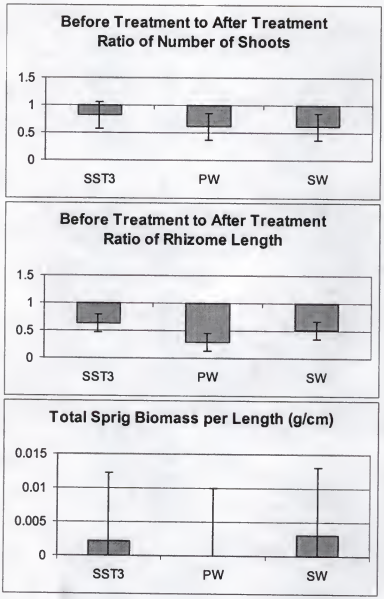


Figure 4-26: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and total sprig biomass (bottom panel) for *Ruppia* in Experiment 3. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

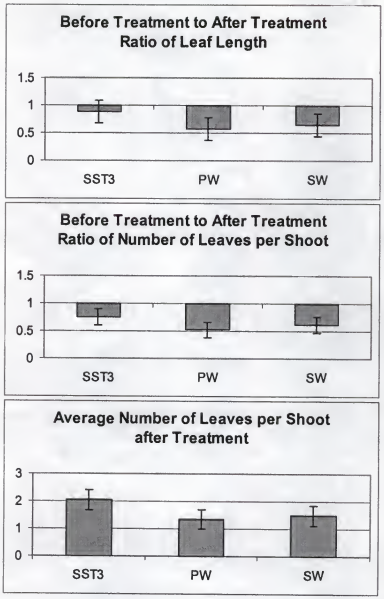


Figure 4-27: Leaf characteristics for *Ruppia* after Experiment 3. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Table 4-6: Averages and standard deviations of morphometrics measured on *Ruppia* sprigs prior to and after experimental treatments.

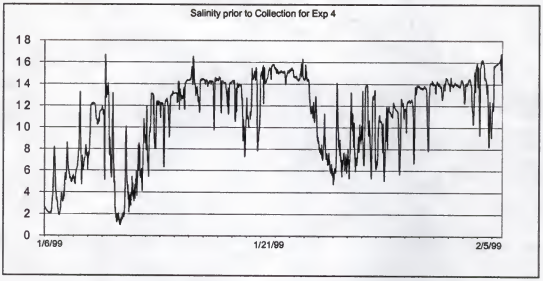
	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SST3	12.54	6.17	8.96	5.45
PW	9.96	4.13	6.23	5.95
SW	12.13	5.83	6.33	5.83
Rhizome Length (cm)				
SST3	13.17	6.18	7.52	5.90
PW	11.08	5.08	3.42	3.91
SW	12.13	4.88	5.79	5.82
Leaf length (cm)				
SST3	3.94	1.16	3.32	1.34
PW	3.96	1.08	2.10	1.48
SW	3.92	0.86	2.49	1.48
Leaf Number				
SST3	2.69	0.45	1.94	0.96
PW	2.57	0.49	1.28	0.80
SW	2.57	0.77	1.49	0.92

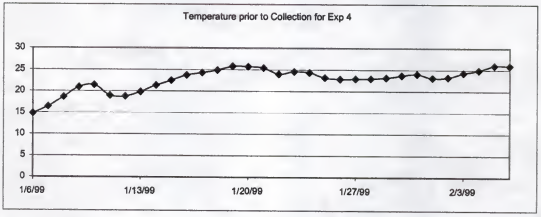
Experiment 4: Salinity Fluctuation around Different Means

The overall effect of salinity fluctuation was less on *Thalassia* and *Halodule* when salinities fluctuated at higher salinities. *Ruppia* had similar responses to both treatments, although increases in leaf length and number were greater when salinity fluctuated within the less saline range.

Overview of Physical Measurements

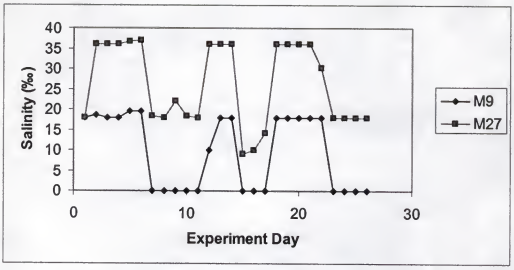
Salinity in Little Madeira Bay during the month prior to collection averaged around 13‰, temperature at 22.7° C (Figure 4-28). The salinity pattern of the two treatments is given in Figure 4-29. The treatments are coded as follows: 1) M9- square wave with an eight day period, an amplitude of 9‰, oscillating around a mean of 9‰ (ranging between 0 and 18‰), and 2) M27- square wave with an eight day period, an

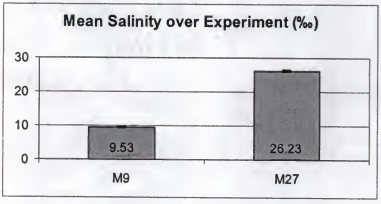




	Salinity (‰)	Temperature (°C)
Mean	13.12	22.7
Standard Deviation	2.4	2.87
Minimum	1.99	12.84
Maximum	17.95	27.35
Measured at Collection	16	26

Figure 4-28: Salinity (top panel) and temperature (middle panel) in Little Madeira Bay a month prior to seagrass collection (Data from Patino and Hittle, unpublished). Salinity values were measured at 15- minute intervals. Temperature values are daily averages. Tabular values (bottom panel) are based on data collected at 15-minute intervals (except for data collected at time of collection).





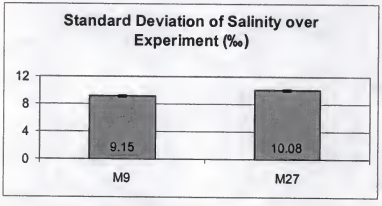


Figure 4-29: Salinity patterns for treatments in Experiment 4. (Coding as follows: 1) T9- Square wave with amplitude of 9‰, period of eight days, oscillating around 9‰, and 2) T27- Square wave with amplitude of 9‰, period of eight days, oscillating around 27‰)

amplitude of 9‰, oscillating around a mean of 27‰ (ranging between 18 and 36). Although the design called for square waves with eight day periods, pump problems made it necessary to run a wave pattern with a ten day, six day, and finally eight day period. Both treatments followed this pattern.

The standard deviation of salinity in the T27 treatment was higher than that of the T9 treatment (Figure 4-29, bottom panel) due to an unanticipated salinity drop on Day 15. The more saline water in the T27 treatment was warmer, clearer, had a greater percent of light reaching bottom, and therefore a significantly greater intensity of light at the seagrass level (Figure 4-30). The low salinity treatments received higher concentrations of phosphorus, due to elevated concentrations in the freshwater supply, whereas those in the higher salinity treatment received slightly higher concentrations of total nitrogen (Figure 4-31).

Thalassia Measurements

Salinity fluctuation affected *Thalassia* in this experiment, however the affect was lessened when salinity fluctuated around a higher mean. The number of shoots as well as the number of leaves per shoot increased in the high salinity treatments.

Green-leaf indices of *Thalassia* decreased over the course of the experiment in both treatments, although the decline was greater in the less saline treatment (Figure 4-32). An increase in GLI occurred in latter third of the more saline treatment.

Before to after treatment ratios of the number of shoots and rhizome lengths after the experiment were slight and varied little between the treatments (Figure 4-33, top and middle panel). The greater belowground biomass in *Thalassia* in the less saline treatment was not statistically significant (Figure 4-33, bottom panel). Average leaf length

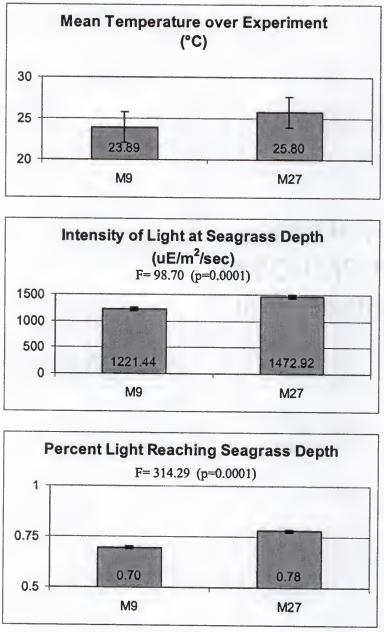


Figure 4-30: Mean temperature (top panel), light intensity (middle panel) and percent light reaching depth of seagrass (bottom panel) for treatments in Experiment 4. Error bars are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time. F ratios are given when differences between means are significant. Percent is expressed in decimal form.

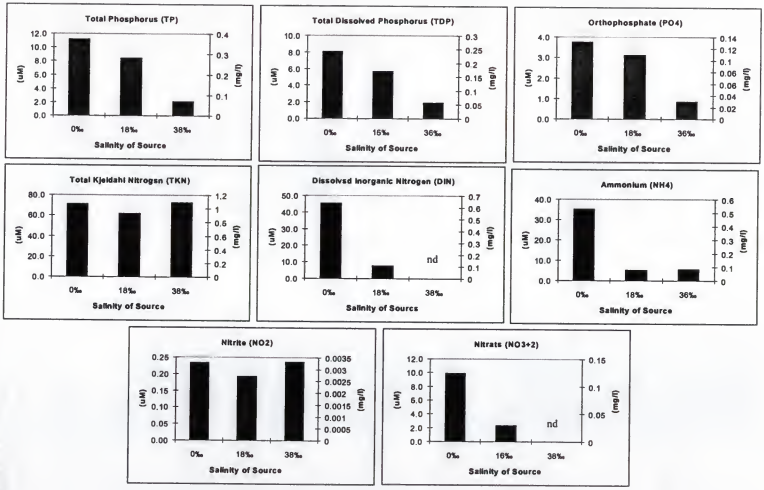
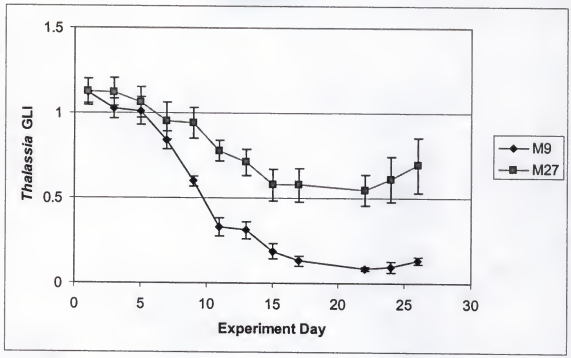


Figure 4-31: Mean nutrient concentrations measured during Experiment 4. (nd- no data)



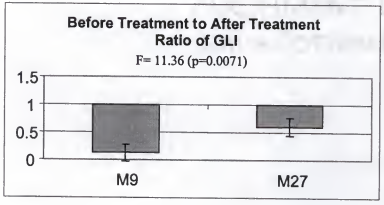


Figure 4-32: Green-leaf indices for *Thalassia* over Experiment 4 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

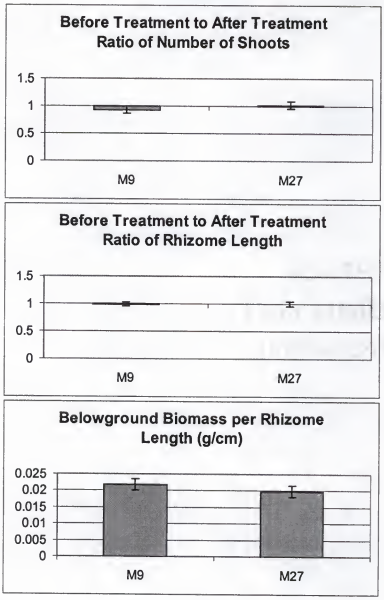


Figure 4-33: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and belowground biomass (bottom panel) for *Thalassia* in Experiment 4. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

decreased less in the more saline treatment, and the number of leaves increased by an average of over 20% (Figure 4-34, top and second panel). *Thalassia* sprigs in the less saline treatment ended with approximately 40% fewer leaves per shoot. Aboveground structures in the less saline treatment had more biomass, however (Figure 4-34, bottom panel). Average shoot, rhizome, and leaf measurements, and the standard deviations of the measurements, are given in Table 4-7.

Halodule Measurements

Overall declines in *Halodule* GLI were also observed in both treatments (Figure 4-35). In the more saline treatment, *Halodule* GLI increased during the initial third of the experiment. During this time the GLI of sprigs in the less saline treatment sharply declined. By Day 15, GLI reached its lowest level in the less saline treatment, where it remained for the remainder of the experiment.

The number of *Halodule* shoots in the more saline treatment decreased less after treatment than those in the lower salinity treatment (Figure 4-36, top panel). Rhizome length increased in the higher salinity treatment as well (Figure 4-36, middle panel). Sprig biomass was significantly greater in the less saline treatment than those in the more saline treatment, however (Figure 4-36, bottom panel).

Leaf lengths declined by about the same amount in both treatments (Figure 4-37, top panel). *Halodule* shoots had an average of 14% more leaves after the more saline treatment in contrast to the 32% decrease in the less saline treatment (Figure 4-37, middle panel). On average, *Thalassia* and *Halodule* had an additional leaf per shoot in the more saline treatment (Figure 4-34, third panel, Figure 4-37, bottom panel). The average number of leaves was statistically significant higher for *Thalassia* and *Halodule* in the

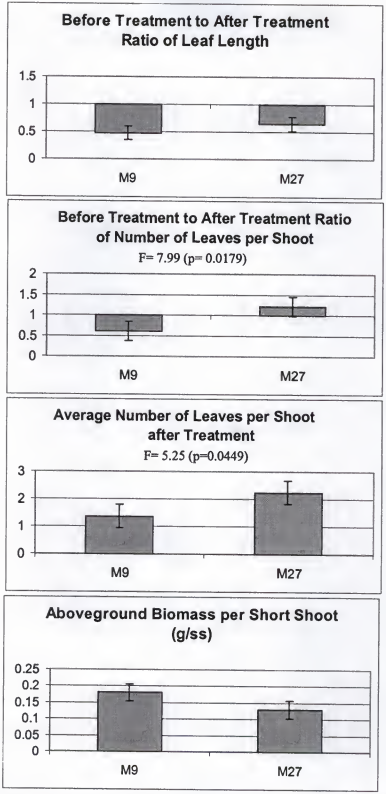


Figure 4-34: Leaf characteristics for *Thalassia* after Experiment 4. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Table 4-7: Averages and standard deviations of morphometrics measured on *Thalassia* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
M9	4.39	1.23	4.03	1.30
M27	3.92	1.23	3.92	1.18
Rhizome Length (cm)				
M9	33.11	13.52	33.43	11.21
M27	28.15	8.87	28.06	8.48
Leaf length (cm)				
M9	18.27	4.50	8.19	7.42
M27	18.37	4.75	11.34	4.93
Leaf Number				
M9	2.39	0.60	1.42	1.20
M27	2.33	0.86	2.53	2.06

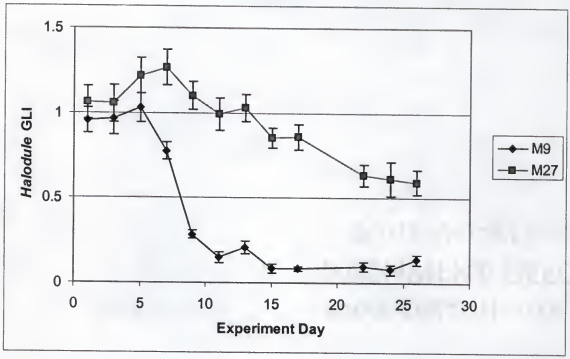
higher salinity treatment. Average shoot, rhizome, and leaf measurements for *Halodule*, and the standard deviations of the measurements, are given in Table 4-8.

Ruppia Measurements

Ruppia varied little in GLI between the two treatments, in sharp contrast to the other two species (Figure 4-38). Green-leaf index increased in both treatments over the experiment. The increase in the less saline treatment was slightly larger.

Increases in *Ruppia* shoot number were similar between treatments (Figure 4-39, top panel). The increase in *Ruppia* rhizome length was greater in the more saline treatment but not significantly so (Figure 4-39, middle panel). Total sprig biomass was greater in the less saline treatment (Figure 4-39, bottom panel).

Leaf length changed little in either treatment (Figure 4-40, top panel). The number of leaves per shoot increased by 5% in the less saline treatment, but declined by



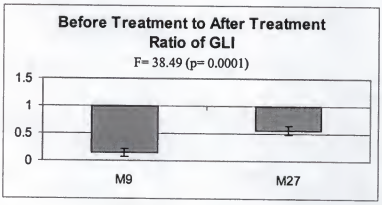


Figure 4-35: Green-leaf indices for *Halodule* over Experiment 4 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

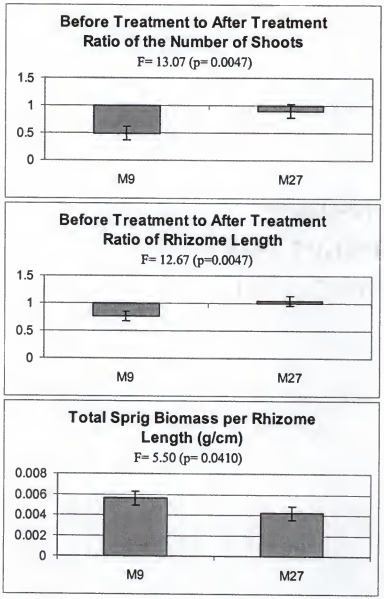


Figure 4-36: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and total sprig biomass (bottom panel) for *Halodule* in Experiment 4. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

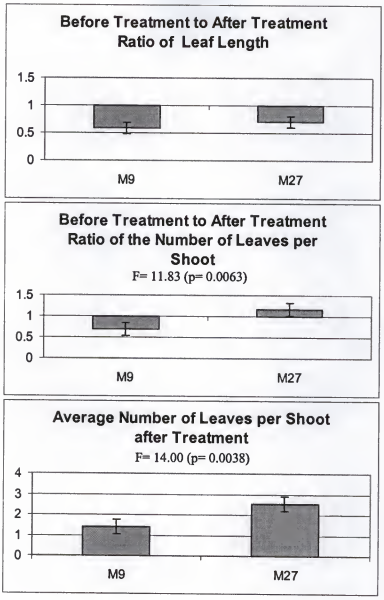
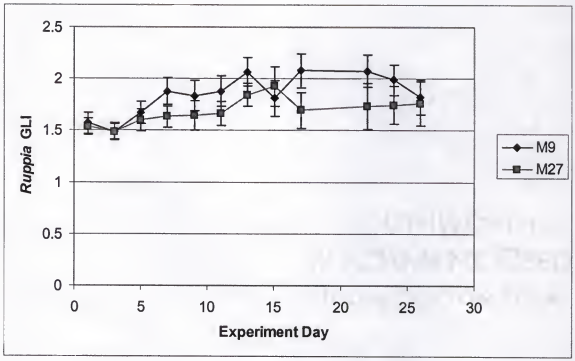


Figure 4-37: Leaf characteristics for *Halodule* after Experiment 4. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Table 4-8: Averages and standard deviations of morphometrics measured on *Halodule* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
M9	4.89	1.33	2.42	1.61
M27	4.19	1.09	3.75	1.90
Rhizome Length (cm)				
M9	12.03	5.19	9.23	5.32
M27	10.74	4.54	10.77	4.75
Leaf length (cm)				
M9	7.24	1.84	3.86	2.09
M27	8.42	2.05	5.61	0.93
Leaf Number				
M9	2.25	0.50	1.53	0.97
M27	2.25	0.44	2.56	0.65

8% in the more saline treatment (Figure 4-40, middle panel). The number of *Ruppia* leaves per shoot, however, was similar in both treatments (Figure 4-40, bottom panel). Average shoot, rhizome, and leaf measurements, and the standard deviations of the measurements, are given in Table 4-9.



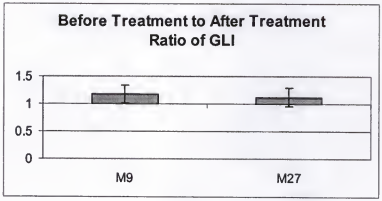


Figure 4-38: Green-leaf indices for *Ruppia* over Experiment 4 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

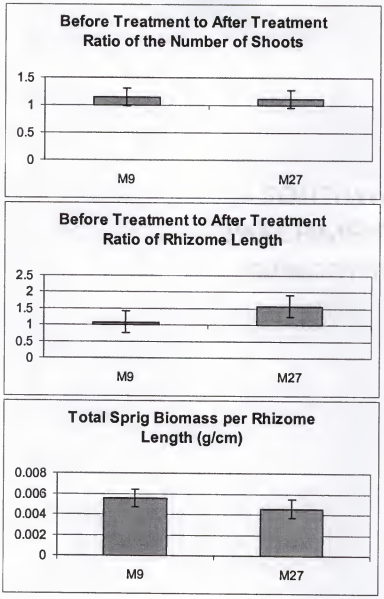


Figure 4-39: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and total sprig biomass (bottom panel) for *Ruppia* in Experiment 4. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

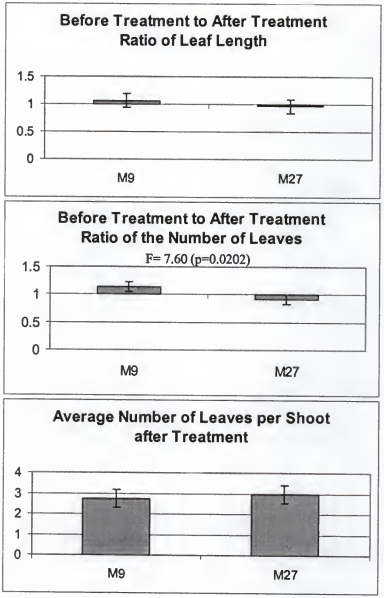


Figure 4-40: Leaf characteristics for *Ruppia* after Experiment 4. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Table 4-9: Averages and standard deviations of morphometrics measured on *Ruppia* sprigs prior to and after experimental treatments.

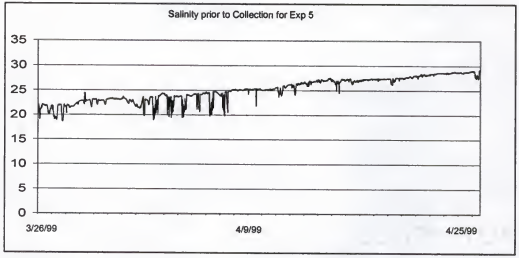
	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
M9	13.61	5.53	15.33	7.65
M27	15.97	7.44	18.61	12.31
Rhizome Length (cm)				
M9	11.33	5.13	11.81	6.60
M27	14.28	6.71	21.46	18.68
Leaf length (cm)				
M9	4.35	0.50	4.59	0.73
M27	4.61	0.77	4.45	1.88
Leaf Number				
M9	2.97	0.84	3.11	0.46
M27	3.22	0.72	2.97	1.30

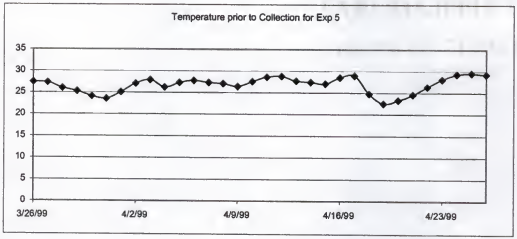
Experiment 5: Extreme Salinity Fluctuation and Effect of Period

Salinity fluctuation had a profound effect on *Thalassia* and *Halodule* in this experiment, regardless of the period (frequency) of the salinity fluctuation treatment pattern. Once again, *Ruppia* was the most resilient, although the high frequency salinity fluctuation treatment was the most detrimental.

Overview of Physical Measurements

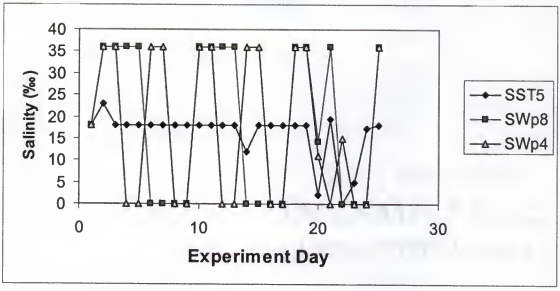
Salinity gradually increased in Little Madeira Bay over the month prior to collection, averaging around 25‰ (Figure 4-41). The salinity pattern for the three treatments is given in Figure 4-42. The three treatments are coded as follows: 1) SST5- stable salinity treatment, 2) SWp8- square wave with amplitude of 18‰ (ranging between 0 and 36‰) and a period of eight days (a salinity change every four days), and 3) SWp4- square wave with amplitude of 18‰ and a period of four days (a salinity change every two days). The square wave period with the eight-day period was plotted

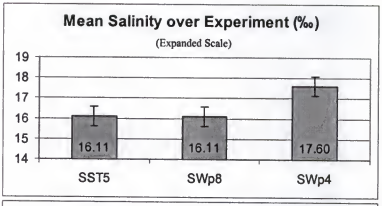




	Salinity (‰)	Temperature (°C)
Mean	25.6	26.84
Standard Deviation	2.37	2.19
Minimum	20.26	20.86
Maximum	29.4	31.82
Measured at Collection	30	29

Figure 4-41: Salinity (top panel) and temperature (middle panel) in Little Madeira Bay a month prior to seagrass collection (Data from Patino and Hittle, unpublished). Salinity values were measured at 15- minute intervals. Temperature values are daily averages. Tabular values (bottom panel) are based on data collected at 15-minute intervals (except for data collected at time of collection).





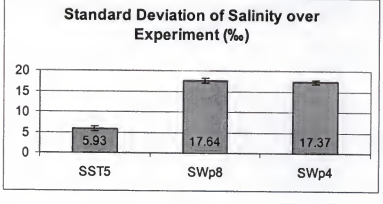


Figure 4-42: Salinity patterns for treatments in Experiment 5. (Coding as follows: 1) SST5- Stable salinity treatment, 2) SWp8- Square wave with amplitude of 18‰ and period of eight days, and 3) SWp4- Square wave with amplitude of 18‰ and period of four days)

before the four-day period treatment, because the frequency of the four-day period is higher, and assumed to be more of stress on the seagrasses. Problems with the saltwater pump occurred during the final week of the experiment, lowering the mean salinity of the treatments and increasing the calculated standard deviation of the stable salinity treatment (Figure 4-42, bottom panel).

The water in the tanks of the low frequency treatment was clearest: 65% of the light measured at the surface reached seagrass level (Figure 4-43, bottom panel). Seagrass in the low frequency treatment received the greatest intensity of light, followed by those in the high frequency and stable salinity treatments (Figure 4-43, middle panel). Nutrient data are provided in Figure 4-44.

Thalassia Measurements

The effect of salinity fluctuation on *Thalassia* was dramatic. Green-leaf index increased in *Thalassia* in the stable salinity treatment over the course of the experiment (Figure 4-45). Although both frequency treatments resulted in the same percent change of GLI, those in the higher frequency treatment (four-day period) had a steeper decline during the initial third of the experiment than those in the lower frequency treatment (eight-day period). Not until Day 15, did both frequency treatments yield similar GLI values. Green-leaf indices for the frequency treatments were 2% of their initial values at the end of the experiment (Figure 4-45, bottom panel)

The number of *Thalassia* shoots decreased in both frequency treatments, whereas in the stable salinity treatment, shoot number increased by 10% (Figure 4-46, top panel). Rhizome length did not change considerably in any treatment (Figure 4-46, middle panel). Rhizome biomass was least in the high frequency (SWp4) treatment (Figure 4-

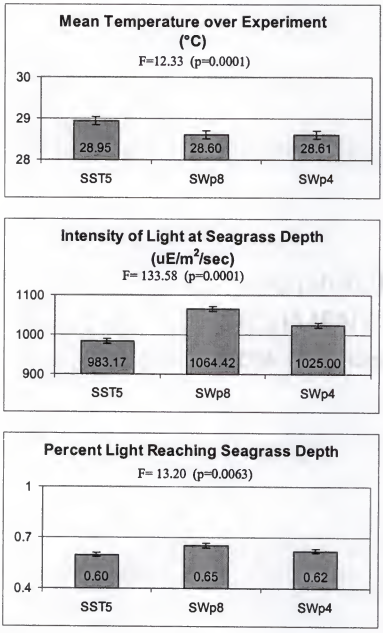


Figure 4-43: Mean temperature (top panel), light intensity (middle panel), and percent light reaching depth of seagrass (bottom panel) for treatments in Experiment 5. Error bars are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time. F ratios are given when differences between means are significant. Percent is expressed in decimal form.

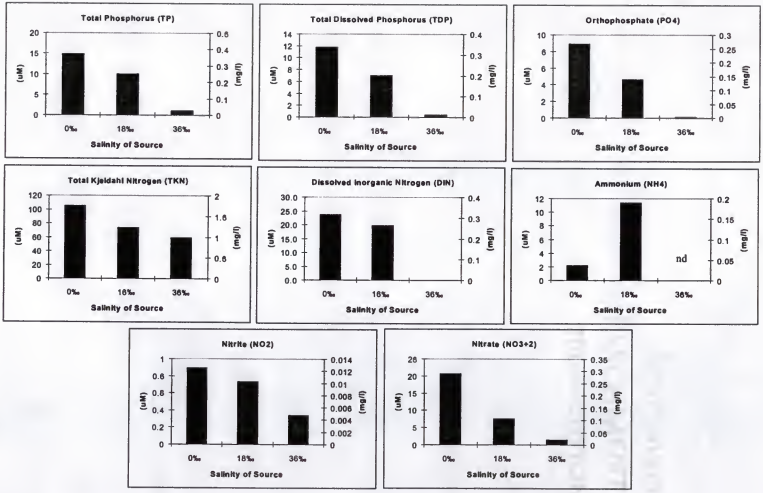
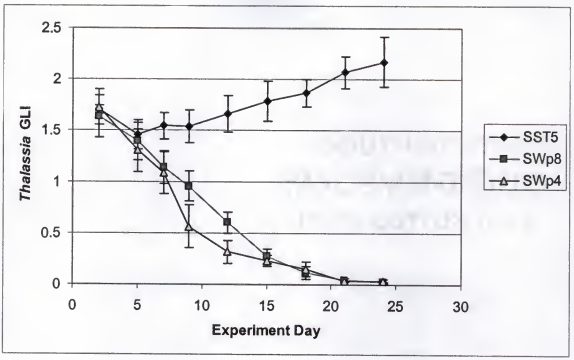


Figure 4-44: Mean nutrient concentrations measured during Experiment 5. (nd- no data)



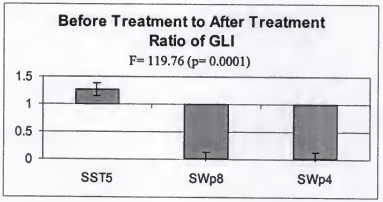


Figure 4-45: Green-leaf indices for *Thalassia* over Experiment 5 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals derived from Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

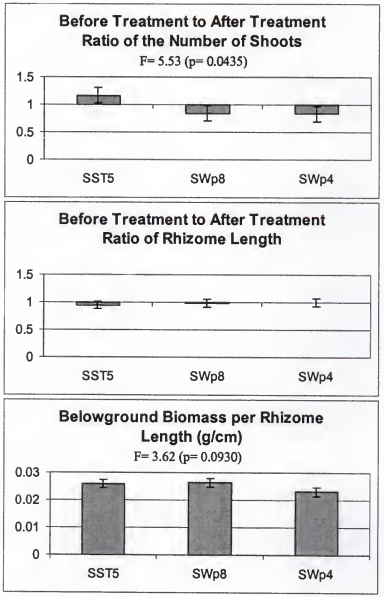


Figure 4-46: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and belowground biomass (bottom panel) for *Thalassia* in Experiment 5. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

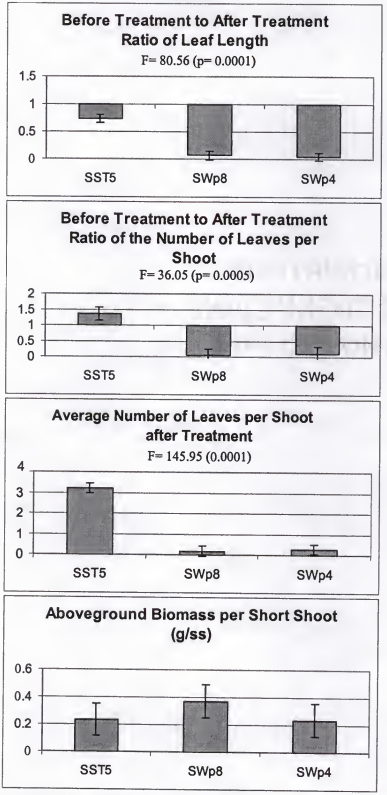


Figure 4-47: Leaf characteristics for *Thalassia* after Experiment 5. All percents are expressed in decimal form. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Table 4-10: Averages and standard deviations of morphometrics measured on *Thalassia* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SST5	3.89	1.68	4.28	1.67
SWp8	3.56	1.38	2.89	1.13
SWp4	3.89	1.57	3.22	1.56
Rhizome Length (cm)				
SST5	37.16	20.04	34.75	18.74
SWp8	30.09	13.01	29.92	13.88
SWp4	32.57	10.01	31.61	8.40
Leaf length (cm)				
SST5	20.33	5.05	14.83	5.31
SWp8	21.12	4.61	1.08	3.25
SWp4	20.54	6.02	0.96	2.79
Leaf Number				
SST5	2.78	0.73	3.22	0.65
SWp8	2.67	0.49	0.17	0.51
SWp4	2.72	0.67	0.28	0.83

46, bottom panel). The leaf length and number of leaves per shoot decreased considerably, yet increased in the stable salinity treatment (Figure 4-47, top and second panel). On average, *Thalassia* in this treatment had over three leaves per shoot after the experiment, whereas those in the four-day and eight-day period treatments had 0.28 and 0.17 leaves per shoot, respectively (Figure 4-47, third panel). Averages and standard deviations of shoot, rhizome, and leaf morphometrics are provided in Table 4-10.

Halodule Measurements

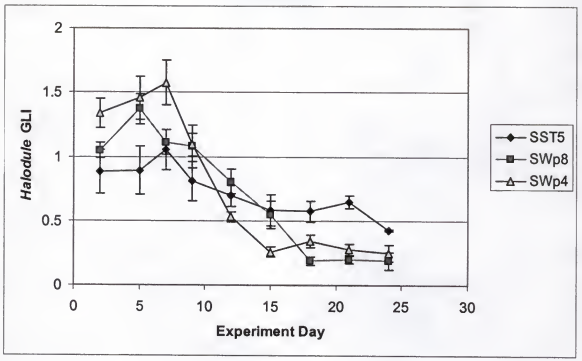
Declines in *Halodule* green-leaf index occurred in all treatments (Figure 4-48). The magnitude of decline was less in the stable salinity treatment, where *Halodule*

retained over 50% of its original green-leaf index, versus less than 20% in both frequency treatments. The beginning GLI of the *Halodule* used in the stable salinity treatment was lower as well, as indicated on the top panel of Figure 4-48. In the frequency treatments, the greatest declines occurred midway through the experiment.

The number of shoots per *Halodule* sprig decreased in all treatments, as did the length of rhizome (Figure 4-49, top and middle panel). Total sprig biomass was least in the high frequency treatment, but was not significantly higher in the other treatments (Figure 4-49, lower panel). The average length of *Halodule* leaves increased in the stable salinity treatment, although the number of leaves per shoot was essentially the same as that prior to treatment (Figure 4-50, top and middle panel). Similar decreases in leaf length and number occurred for plants in both square wave treatments. At the conclusion of the experiment, *Halodule* in the frequency treatments finished with approximately half of the number of leaves per shoot than those in the stable salinity treatment (Figure 4-50, bottom panel). Averages and standard deviations of morphometrics are given in Table 4-11.

Ruppia Measurements

Despite the extreme salinity fluctuation treatments, *Ruppia* was resilient. A decline in *Ruppia* green-leaf index occurred only in the high frequency (SWp4) treatment (Figure 4-51). However, *Ruppia* in this treatment retained over 80% of its initial GLI values. In addition, green-leaf indices increased during the middle segment of the experiment. GLI values of *Ruppia* in the low frequency (SWp8) treatment increased slightly, 10% more than initial values (Figure 4-51, bottom panel).



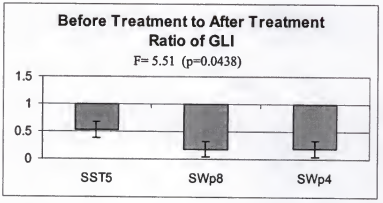


Figure 4-48: Green-leaf indices for *Halodule* over Experiment 5 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

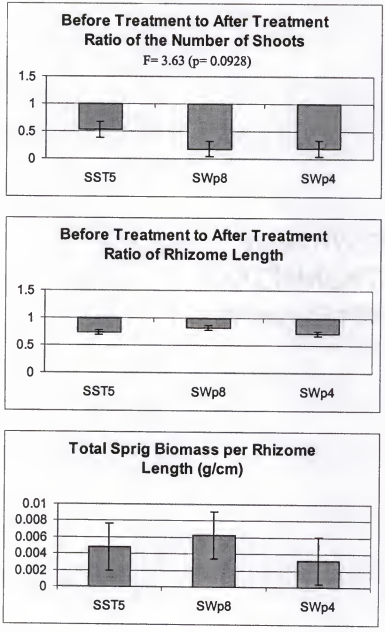


Figure 4-49: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and total sprig biomass (bottom panel) for *Halodule* in Experiment 5. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

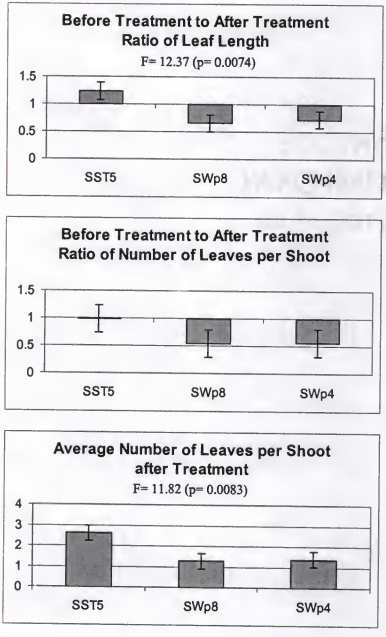
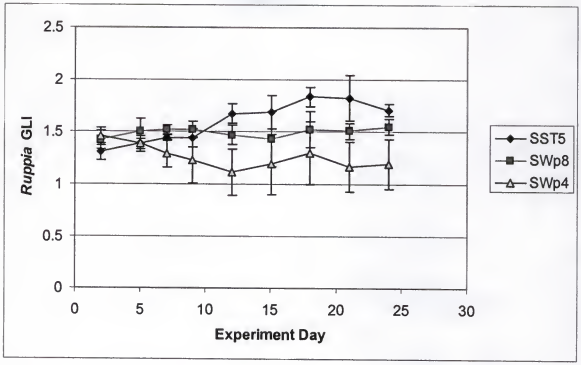


Figure 4-50: Leaf characteristics for *Halodule* after Experiment 5. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Table 4-11: Averages and standard deviations of morphometrics measured on *Halodule* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SST5	5.72	3.25	3.06	1.63
SWp8	5.39	3.13	3.28	1.56
SWp4	6.89	2.22	3.06	2.13
Rhizome Length (cm)				
SST5	16.92	9.68	13.13	10.29
SWp8	15.75	10.42	12.36	7.22
SWp4	22.93	7.85	16.43	8.59
Leaf length (cm)				
SST5	6.54	1.70	7.62	2.25
SWp8	6.41	1.58	3.94	1.96
SWp4	5.94	0.84	4.12	2.15
Leaf Number				
SST5	2.72	0.67	2.61	0.78
SWp8	2.61	0.50	1.28	0.75
SWp4	2.72	0.57	1.39	0.85

The greatest decrease in the number of shoots and rhizome length occurred in the high frequency fluctuation treatment (Figure 4-52, top and middle panels). *Ruppia* in the high frequency treatment had the greatest percent loss of leaves per shoot, as well (Figure 4-53, middle panel). Leaf length increased in all treatments, most in the stable salinity treatment (Figure 4-53, top panel). Morphometric values are given in Table 4-12.



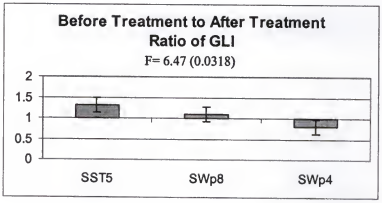


Figure 4-51: Green-leaf indices for *Ruppia* over Experiment 5 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals derived from Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

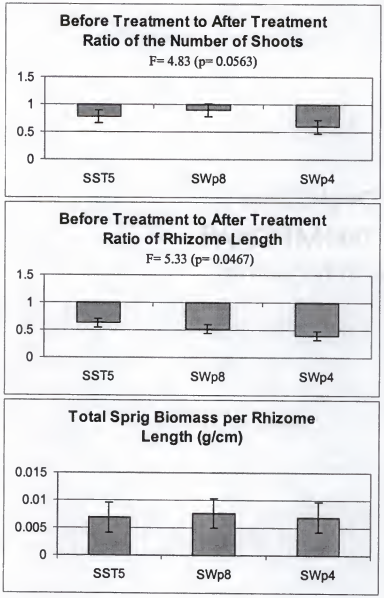


Figure 4-52: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and total sprig biomass (bottom panel) for *Ruppia* in Experiment 5. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

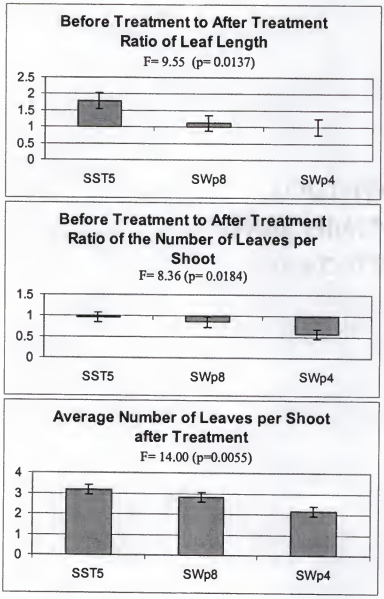


Figure 4-53: Leaf characteristics for *Ruppia* after Experiment 5. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Table 4-12: Averages and standard deviations of morphometrics measured on *Ruppia* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SST5	11.28	5.20	8.11	2.52
SWp8	11.83	3.60	10.22	4.26
SWp4	12.63	6.27	8.31	4.80
Rhizome Length (cm)				
SST5	9.20	5.02	5.06	2.98
SWp8	11.27	5.40	5.59	3.42
SWp4	10.68	8.68	4.41	3.26
Leaf length (cm)				
SST5	4.46	0.72	7.92	1.79
SWp8	4.65	0.77	5.06	0.73
SWp4	4.37	0.84	4.84	1.71
Leaf Number				
SST5	3.39	0.61	3.17	0.51
SWp8	3.35	1.09	2.83	0.38
SWp4	3.81	0.91	2.44	0.96

Experiment 6: Salinity Fluctuation and Circulation: Constant Throughflow of Water vs. Air Circulation in Experimental Tanks

Regardless of the method of water circulation, *Thalassia* was negatively influenced by the salinity fluctuation treatments. *Halodule* was affected by salinity fluctuation as well, however when salinities were stable, air circulation lead to greater biological responses. *Ruppia* responded well in the air circulated tanks as well, despite the exposure to fluctuating salinities.

Overview of Physical Measurements

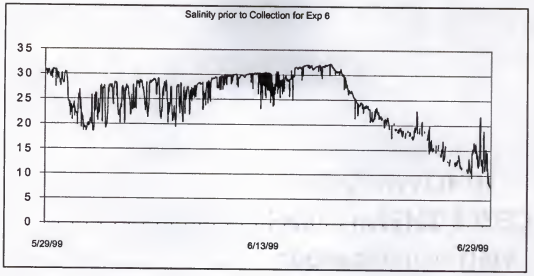
Salinity at the collection site was decreasing in the month prior to collection, from nearly 30 to approximately 15‰ during that time (Figure 4-54). Temperature varied a few degrees around 30° C every 5 to 7 days.

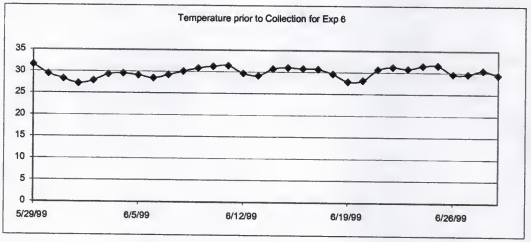
The salinity patterns for the treatments in this experiment are given in Figure 4-55. The treatments are coded as follows: 1) SSTt- stable salinity treatment circulated by constant inflow of water, 2) SSTb- stable salinity treatment circulated by constant bubbling, 3) SWt- square wave with amplitude of 14‰ and eight day period, circulated by constant inflow of water, and 4) SWb- square wave with amplitude of 14‰ and eight day period, circulated by constant bubbling. Problems with the freshwater pump occurred on Days 4 and 16, causing spikes in salinity in the throughflow treatments.

Greater control of salinity was achieved in the bubbled treatments. Mean salinities were approximately 18‰ in the aerated treatments; salinity standard deviation in bubbled stable salinity treatment was an order of magnitude less than that in the throughflow stable salinity treatment (Figure 4-55, bottom panel). Temperature was similar in all treatments, however light intensity and water clarity was higher in the air circulated tanks (Figure 4-56). Nutrient data are provided in Figures 4-57. A noticeable decrease in total phosphorus and total nitrogen concentrations occurred in the freshwater supply relative to that in prior experiments.

Thalassia Measurements

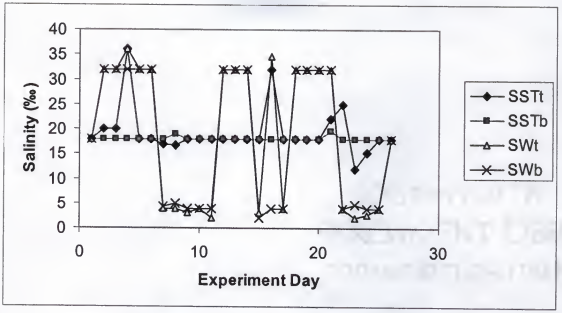
The effect of fluctuation was far more important than the effect of circulation method in spite of the greater water clarity, water movement, and controllability of salinity in the bubbled tanks. Green-leaf indices of *Thalassia* in both the stable salinity

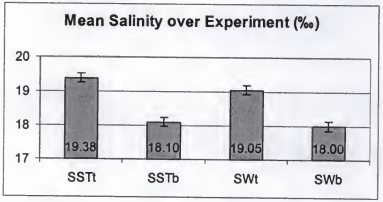




	Salinity (‰)	Temperature (°C)
Mean	26.94	29.78
Standard Deviation	4.53	1.54
Minimum	7.49	26.03
Maximum	32.24	33.3
Measured at Collection	18	30

Figure 4-54: Salinity (top panel) and temperature (middle panel) in Little Madeira Bay a month prior to seagrass collection (Data from Patino and Hittle, unpublished). Salinity values were measured at 15- minute intervals. Temperature values are daily averages. Tabular values (bottom panel) are based on data collected at 15-minute intervals (except for data collected at time of collection).





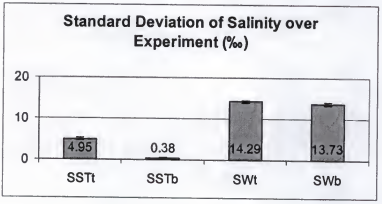


Figure 4-55: Salinity patterns for treatments in Experiment 6. (Coding as follows: 1) SSTt- Stable salinity treatment circulated by constant throughflow of water, 2) SSTb- Stable salinity treatment circulated by air bubbling, 3) SWt- Square wave with amplitude of 14‰, period of eight days, and circulated by throughflow of water, and 4) SWb- Square wave with amplitude of 14‰, period of eight days, and circulated by air bubbling.)

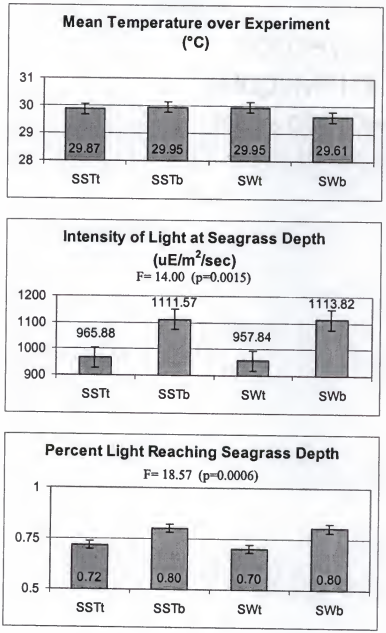


Figure 4-56: Mean temperature (top panel), light intensity (middle panel) and percent light reaching depth of seagrass (bottom panel) for treatments in Experiment 6. Error bars are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time. F ratios are given when differences between means are significant. Percent is expressed in decimal form.

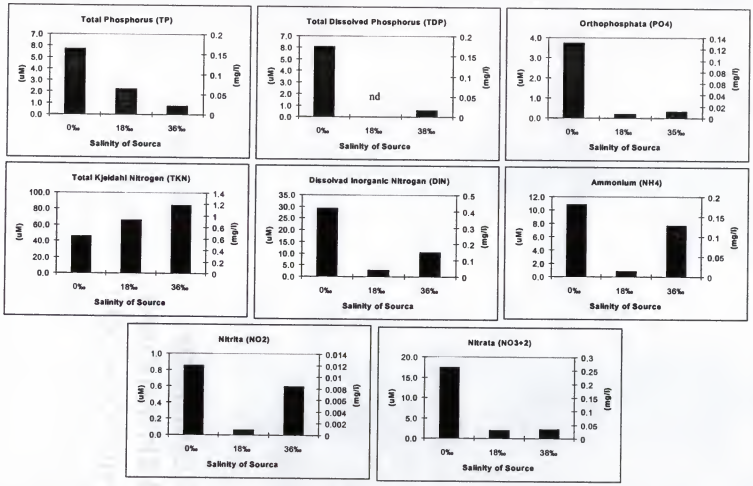


Figure 4-57: Mean nutrient concentrations measured over Experiment 6. (nd- no data)

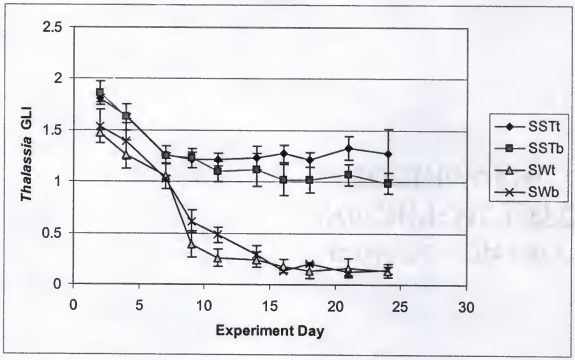
and fluctuation treatments declined during the first third of the experiment, regardless of the method of circulation (Figure 4-58). Following the initial decline, an increase occurred in the stable salinity throughflow treatment (SSTt), where a gradual decline ensued in the stable air circulated treatment (SSTb). Both square wave treatments followed a similar trend throughout the experiment, ending in green-leaf indices of about 10% original values (Figure 4-58, bottom panel).

Shoot number increased in both stable salinity treatments, whereas slight decreases occurred in the square wave treatments (Figure 4-59, top panel). Rhizome length and belowground biomass varied little among treatments (Figure 4-59, middle and bottom panels). Leaf morphometrics for *Thalassia* were affected by salinity treatment but not method of circulation, although those in the air circulated stable salinity treatments decreased less than those in the throughflow treatments (Figure 4-60). Averages and standard deviations of shoot, rhizome, and leaf morphometrics are given in Table 4-13.

Halodule Measurements

Halodule was affected by salinity fluctuation regardless of the circulation method, with declines in GLI after treatment (Figure 4-61). *Halodule* GLI increased in the bubbled stable salinity treatment, increasing 33% over initial values. This is the first treatment in which an increase in *Halodule* green-leaf index occurred. A decline in green-leaf index was measured in the stable throughflow treatment, but not as great as the decline measured in both square wave treatments.

The number of *Halodule* shoots and the length of its rhizomes declined in the fluctuation treatments. *Halodule* in the bubbled stable salinity treatment, however,



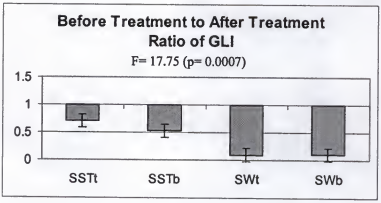


Figure 4-58: Green-leaf indices for *Thalassia* over Experiment 6 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

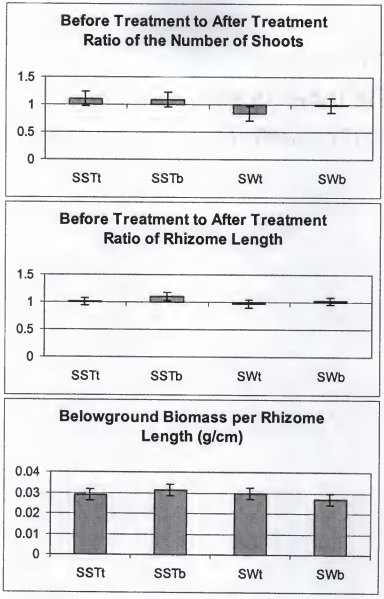


Figure 4-59: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and belowground biomass (bottom panel) for *Thalassia* in Experiment 6. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

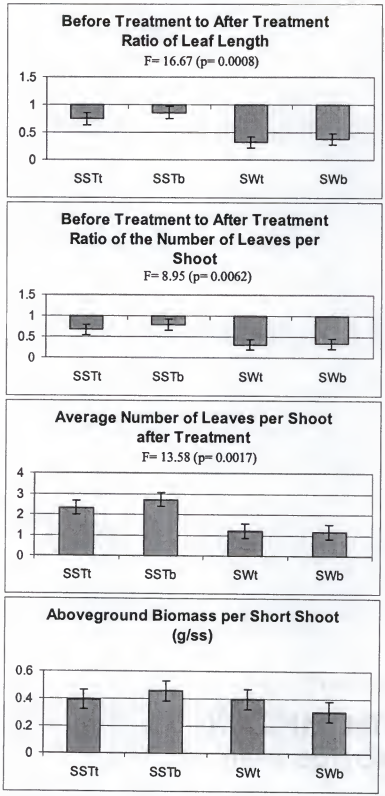
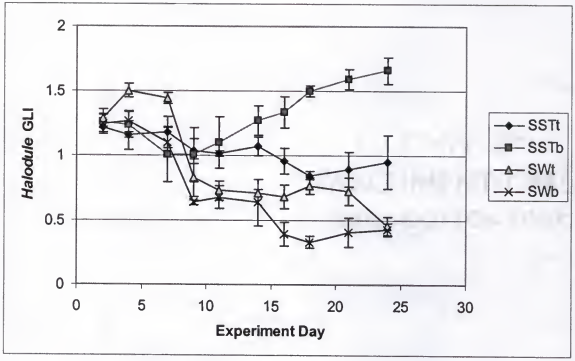


Figure 4-60: Leaf characteristics for *Thalassia* after Experiment 6. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Table 4-13: Averages and standard deviations of morphometrics measured on *Thalassia* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SSTt	3.28	0.83	3.61	1.46
SSTb	3.11	0.90	3.33	1.37
SWt	3.06	0.94	2.39	0.92
SWb	2.89	1.18	2.56	1.38
Rhizome Length (cm)				
SSTt	27.06	7.30	27.15	8.72
SSTb	27.09	9.85	29.55	10.04
SWt	28.60	9.62	27.47	10.15
SWb	25.49	8.32	25.78	8.12
Leaf length (cm)				
SSTt	30.42	5.87	22.30	8.71
SSTb	28.62	6.07	23.98	9.33
SWt	28.59	5.92	8.43	7.34
SWb	27.38	4.98	10.34	9.07
Leaf Number				
SSTt	3.67	0.84	2.33	0.84
SSTb	3.61	0.78	2.72	0.96
SWt	3.89	0.58	1.22	1.17
SWb	3.61	0.78	1.17	1.04

increased in both shoot number and rhizome length, by 69 and 50%, respectively (Figure 4-62, top and middle panels). Declines in leaf length and the number of leaves were similar amongst fluctuation treatments for *Halodule*, although those in the bubbled treatment had less of a decline in leaf length and the greatest percent increase in leaf number (Figure 4-63, top and middle panels). Averages and standard deviations of morphometric measurements are provided in Table 4-14.



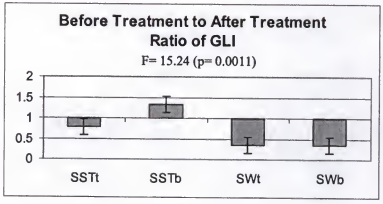


Figure 4-61: Green-leaf indices for *Halodule* over Experiment 6 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

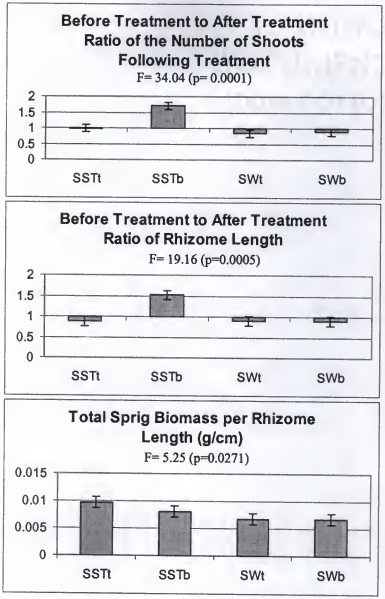


Figure 4-62: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and total sprig biomass (bottom panel) for *Halodule* in Experiment 6. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

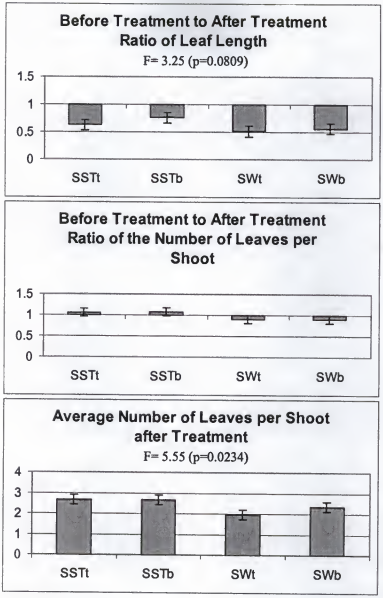


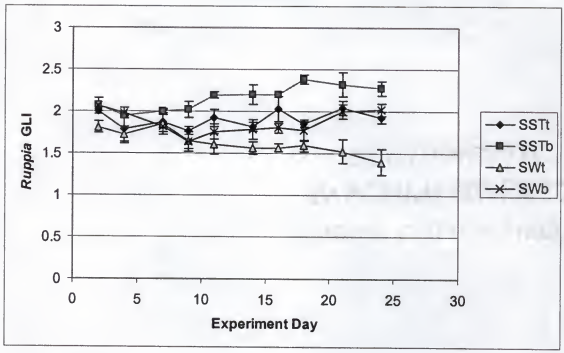
Figure 4-63: Leaf characteristics for *Halodule* after Experiment 6. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Table 4-14: Averages and standard deviations of morphometrics measured on *Halodule* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SSTt	4.89	2.05	4.67	2.22
SSTb	5.17	2.20	8.72	3.97
SWt	4.94	2.60	3.67	1.53
SWb	5.53	2.43	4.94	2.61
Rhizome Length (cm)				
SSTt	17.86	7.94	15.40	9.18
SSTb	17.53	7.19	25.41	9.51
SWt	17.53	11.79	15.40	8.57
SWb	17.82	9.40	16.04	10.42
Leaf length (cm)				
SSTt	12.92	2.91	7.68	1.17
SSTb	12.53	3.23	9.14	2.45
SWt	12.49	3.00	6.44	1.96
SWb	11.72	3.32	6.30	1.02
Leaf Number				
SSTt	2.56	0.51	2.67	0.59
SSTb	2.56	0.51	2.67	0.49
SWt	2.22	0.43	1.94	0.64
SWb	2.65	0.49	2.35	0.49

Ruppia Measurements

The method of circulation seemed to be more of a factor than salinity fluctuation in determining GLI values for *Ruppia.* Similar to *Halodule, Ruppia* green-leaf index increased in the bubbled stable salinity treatment (Figure 4-64). A minimal percent decline in GLI occurred in the throughflow stable salinity treatment, slightly less than that in the bubbled square wave treatment, and not statistically different than declines in the throughflow square wave treatment.



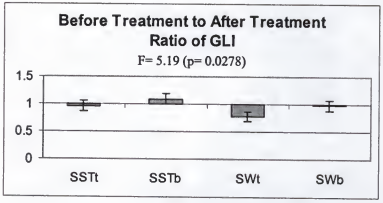


Figure 4-64: Green-leaf indices for *Ruppia* over Experiment 6 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals derived from Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

The largest increases in shoot number and rhizome length were measured on sprigs of *Ruppia* exposed to the bubbled treatments (Figure 4-65, top and middle panels). The greater whole sprig biomasses measured in these treatments, however, were not statistically significant. Leaf responses were not significantly different regardless of circulation method or salinity fluctuation (Figure 4-66, top and middle panels). Averages and standard deviations of *Ruppia* shoot, rhizome, and leaf morphometrics are given in Table 4-15.

Table 4-15: Averages and standard deviations of morphometrics measured on *Ruppia* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SSTt	13.35	5.00	13.12	3.37
SSTb	15.94	4.75	29.78	18.53
SWt	13.39	3.60	11.28	5.60
SWb	12.72	4.62	19.00	6.20
Rhizome Length (cm)				
SSTt	21.29	8.72	18.09	8.12
SSTb	28.18	9.62	47.24	26.17
SWt	23.41	10.24	19.47	10.08
SWb	23.17	6.96	24.88	8.42
Leaf length (cm)				
SSTt	4.99	0.78	5.74	0.81
SSTb	5.28	0.61	4.93	0.99
SWt	4.87	0.66	4.53	1.20
SWb	5.16	0.71	4.09	0.54
Leaf Number				
SSTt	3.41	0.51	3.41	0.51
SSTb	3.33	0.49	3.17	0.38
SWt	3.22	0.65	2.83	0.86
SWb	3.28	0.57	3.06	0.24

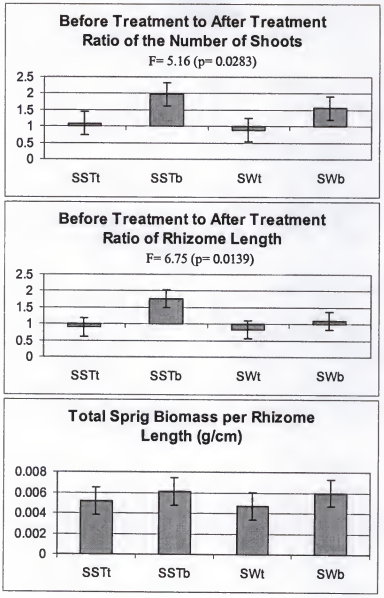


Figure 4-65: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and total sprig biomass (bottom panel) for *Ruppia* in Experiment 6. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

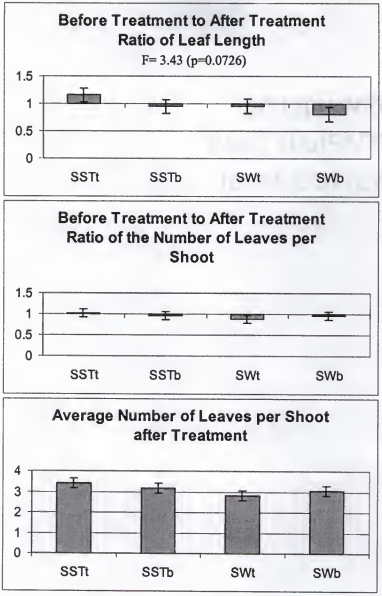


Figure 4-66: Leaf characteristics for *Ruppia* after Experiment 6. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

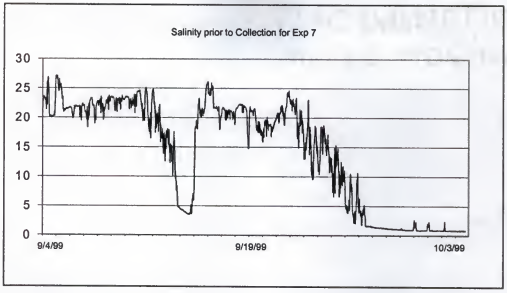
Experiment 7: Effect of Salinity Fluctuation and Reduction of Light

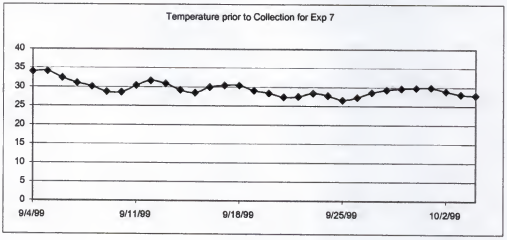
Salinity fluctuation was more of an influence on seagrass survival than light availability. Despite shading to 30% of ambient light, *Thalassia* and *Halodule* green-leaf indices were greater in the stable salinity treatment than those exposed even to the low amplitude fluctuations in full sun. Exposure to high amplitude fluctuation treatments resulted in even more reduced GLI, shoot number, and leaves per shoot for both species.

Overview of Physical Measurements

Salinity at the collection site plummeted approximately 25‰ ten days prior to collection (Figure 4-67, top panel). Temperatures descended during this month, ranging between 26 and 35°C (Figure 4-67, middle panel).

Salinity patterns for the five treatments are given in Figure 4-68. The treatments are coded as follows: 1) SSTs- stable salinity treatment shaded by 70% shadecloth, 2) SWa7u- square wave treatment with amplitude of 7‰, period of eight days, and unshaded, 3) SWa7s- square wave treatment with amplitude of 7‰, period of eight days, and shaded by 70% shadecloth, 4) SWa14u- square wave treatment with amplitude of 14‰, period of eight days, and unshaded and, 5) SWa14u- square wave treatment with amplitude of 14‰, period of eight days, and shaded by 70% shadecloth. Control over salinity from the use of bubble circulation in all treatments is evidenced by the calculated zero standard deviation of salinity in the shaded stable salinity treatment (Figure 4-68, bottom panel). Mean temperatures differed by only 0.13° C amongst tanks, and as designed, light intensities were significantly different between the shaded and unshaded tanks (Figure 4-69). Nutrient data are provided in Figure 4-70.





	Salinity (‰)	Temperature (°C)
Mean	15.78	29.5
Standard Deviation	9.62	1.91
Minimum	0.8	25.94
Maximum	28.04	35.38
Measured at Collection	7	28

Figure 4-67: Salinity (top panel) and temperature (middle panel) in Little Madeira Bay a month prior to seagrass collection (Data from Patino and Hittle, unpublished). Salinity values were measured at 15-minute intervals. Temperature values are daily averages. Tabular values (bottom panel) are based on data collected at 15-minute intervals (except for data collected at time of collection).

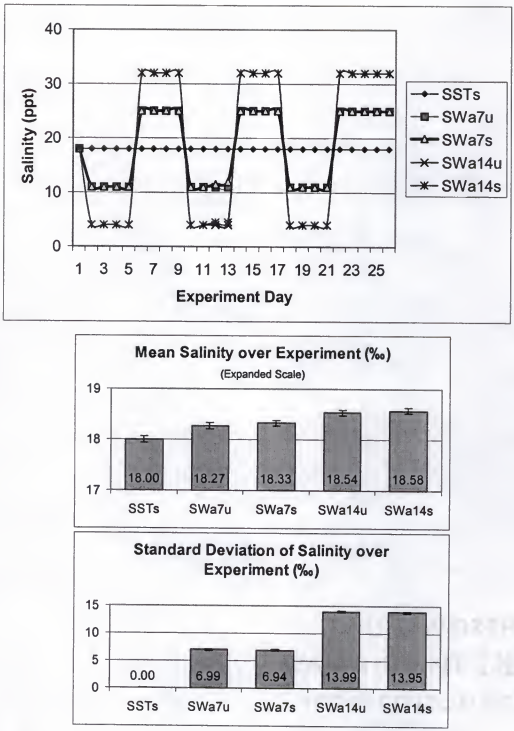


Figure 4-68: Salinity patterns for treatments in Experiment 7. (Coding as follows: 1) SSTs- Stable salinity treatment shaded by 70% shadecloth, 2) SWa7u- Square wave with amplitude of 7, period of eight days and unshaded, 3) SWa7s- Square wave with amplitude of 7, period of eight days and shaded by 70%, 4) SWa14u- Square wave with amplitude of 14, period of eight days and unshaded, and 5) SWa14s- Square wave with amplitude of 14, period of eight days and shaded by 70%.)

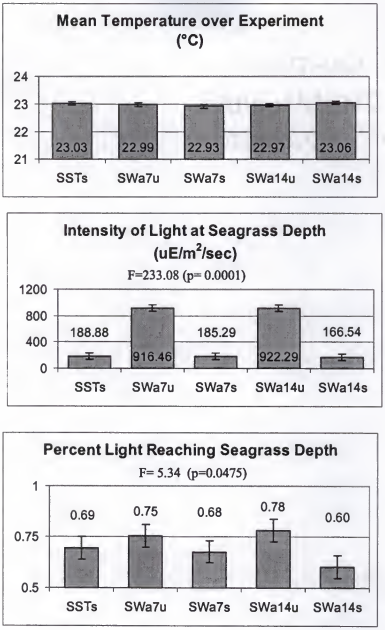


Figure 4-69: Mean temperature (top panel), light intensity (middle panel) and percent light reaching depth of seagrass (bottom panel) for treatments in Experiment 7. Error bars are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time. F ratios are given when differences between means are significant. Percent is expressed in decimal form.

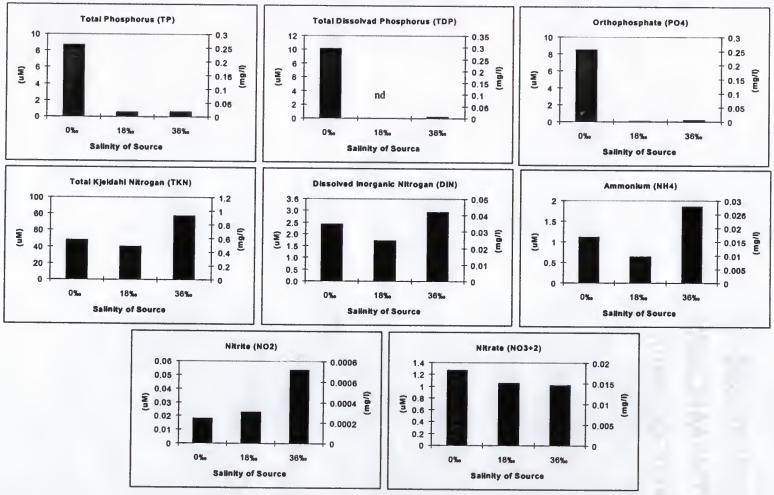


Figure 4-70: Mean nutrient concentrations measured during Experiment 7. (nd- no data)

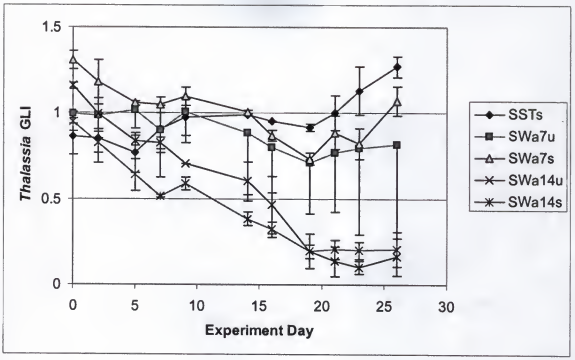
Thalassia Measurements

Salinity fluctuation was more of factor in *Thalassia* green-leaf index than light. GLI measured in the high amplitude treatments decreased throughout the experiment (Figure 4-71). Indices in the stable salinity treatment increased throughout the experiment, whereas those in the low amplitude treatments increased only during the final third. Amongst similar amplitude treatments, shaded treatments fared slightly better, although the differences were not statistically significant.

The number of shoots per sprig declined on those exposed to the high amplitude salinity treatments (Figure 4-72, top panel). Declines in rhizome length occurred only in the unshaded high amplitude treatment (4-72, middle panel). The numbers of leaves per shoot increased in *Thalassia* within the stable salinity treatment (Figure 4-73, top panel). The lower amount of defoliation in the shaded high amplitude treatment was not significantly different in the unshaded high amplitude treatment. Greatest shoot biomass was measured in the high amplitude square wave plants (Figure 4-73, bottom panel). Averages and standard deviations of *Thalassia* morphometric measurements are provided in Table 4-16.

Halodule Measurements

Like *Thalassia, Halodule* green-leaf indices responded to the salinity fluctuation treatment nearly the same way, regardless of light (Figure 4-74). All treatments followed a similar pattern in GLI until Day 19, where green-leaf indices in plants in the shaded stable salinity treatment increased.



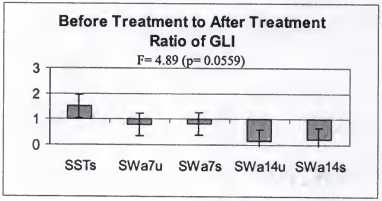


Figure 4-71: Green-leaf indices for *Thalassia* over Experiment 7 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

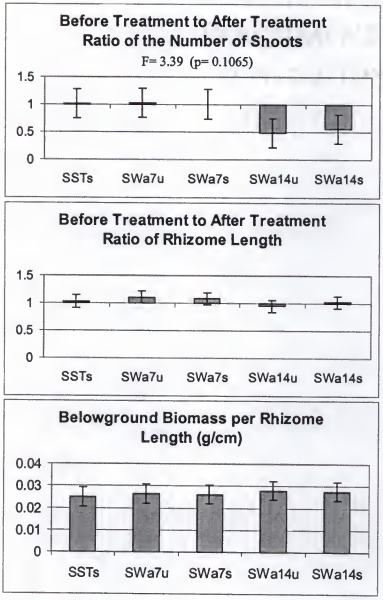


Figure 4-72: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel) and belowground biomass (bottom panel) for *Thalassia* in Experiment 7. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

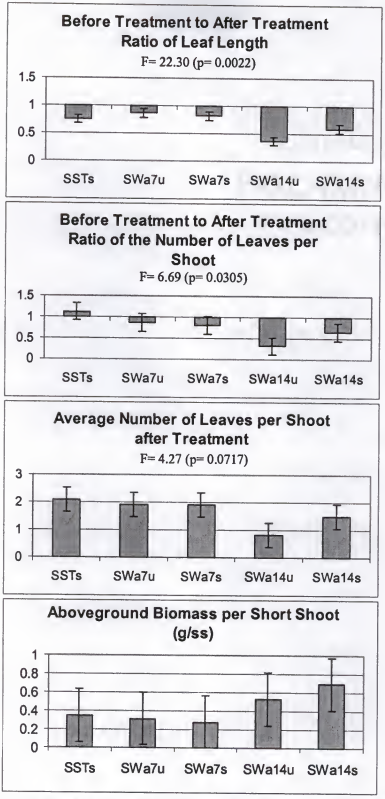
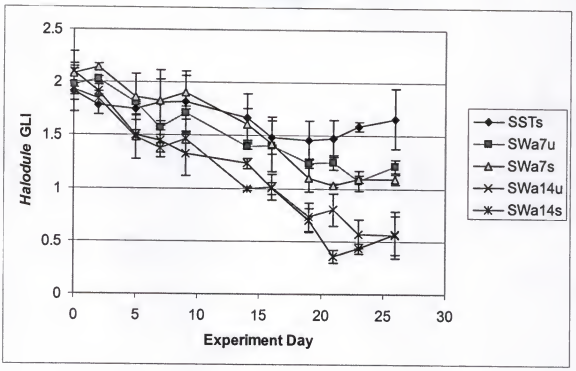


Figure 4-73: Leaf characteristics for *Thalassia* after Experiment 7. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Table 4-16: Averages and standard deviations of morphometrics measured on *Thalassia* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SSTs	2.42	0.51	2.42	0.90
SWa7u	2.92	0.67	2.58	0.90
SWa7s	2.75	0.62	2.75	0.62
SWa14u	2.75	0.75	1.33	1.30
SWa14s	2.92	1.38	1.58	1.38
Rhizome Length (cm)				
SSTs	27.93	8.47	28.77	8.84
SWa7u	28.48	8.91	28.22	7.99
SWa7s	25.12	9.92	26.82	10.35
SWa14u	27.08	17.51	22.40	8.03
SWa14s	35.16	12.50	35.68	12.02
Leaf length (cm)				
SSTs	23.55	4.98	17.58	9.50
SWa7u	19.08	3.82	17.68	4.53
SWa7s	20.88	3.12	17.30	5.31
SWa14u	20.26	3.31	7.71	7.19
SWa14s	21.18	3.04	12.42	6.89
Leaf Number				
SSTs	2.00	0.43	2.08	1.31
SWa7u	2.25	0.87	1.75	0.62
SWa7s	2.50	0.52	1.92	0.79
SWa14u	2.75	1.14	0.83	0.72
SWa14s	2.33	0.49	1.50	1.00

The number of shoots per sprig increased in *Halodule* in the stable salinity and low amplitude treatments (Figure 4-75, top panel). Rhizome length also increased in these treatments (Figure 4-75, middle panel). *Halodule* leaf length and numbers were greater in the low amplitude shaded treatment (SWa7s) (Figure 4-76, top and middle panel). Morphometric values are given in Table 4-17.



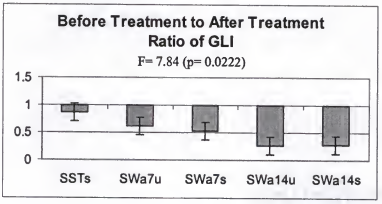


Figure 4-74: Green-leaf indices for *Halodule* over Experiment 7 (top panel) and before to after treatment ratios (bottom panel). Error bars on time series chart represent standard error, those on percent change chart are intervals based on Fisher's least significant difference (LSD) procedure. If the means are not significantly different, the intervals will overlap 95% of the time.

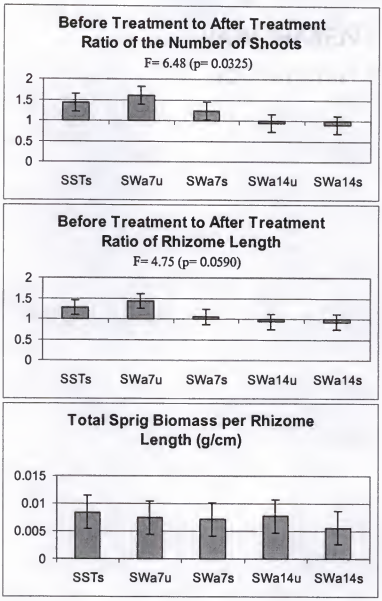


Figure 4-75: Before to after treatment ratios of shoot number (top panel) and rhizome length (middle panel), and total sprig biomass (bottom panel) for *Halodule* in Experiment 7. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

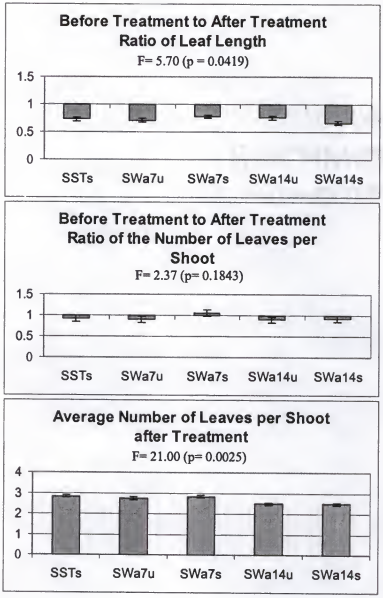


Figure 4-76: Leaf characteristics for *Halodule* after Experiment 7. Intervals around means are based on Fisher's LSD procedure ($p < 0.05$). F ratios are given when differences between means are significant.

Table 4-17: Averages and standard deviations of morphometrics measured on *Halodule* sprigs prior to and after experimental treatments.

	Pre-Experiment		Post-Experiment	
	Average	Std. Dev.	Average	Std. Dev.
Shoot Number				
SSTs	4.67	2.71	6.00	2.92
SWa7u	5.33	2.10	8.17	2.89
SWa7s	4.50	1.51	5.33	1.97
SWa14u	5.00	2.17	4.67	2.71
SWa14s	3.92	0.67	3.50	1.51
Rhizome Length (cm)				
SSTs	16.86	7.29	20.34	8.08
SWa7u	16.70	9.66	23.20	10.63
SWa7s	18.57	6.93	19.24	6.80
SWa14u	20.33	10.75	18.12	10.31
SWa14s	16.20	5.50	14.84	5.67
Leaf length (cm)				
SSTs	11.92	2.49	8.52	1.36
SWa7u	12.20	2.28	8.38	1.15
SWa7s	11.38	2.77	8.73	2.01
SWa14u	10.86	2.13	7.92	1.15
SWa14s	11.53	2.37	7.59	1.47
Leaf Number				
SSTs	3.08	0.29	2.83	0.39
SWa7u	3.17	0.58	2.75	0.45
SWa7s	2.75	0.45	2.83	0.39
SWa14u	2.92	0.67	2.50	0.52
SWa14s	2.75	0.45	2.50	0.52

CHAPTER 5
CORRELATIONS BETWEEN BIOLOGICAL MEASUREMENTS AND PHYSICAL VARIABLES IN FACILITY EXPERIMENTS

Correlations with Salinity

All correlations between *Thalassia* green-leaf indices and salinity fluctuation were negative. Changes in green-leaf indices following treatment in *Thalassia* correlated significantly with all descriptors except mean salinity and number of changes per day (Table 5-1). The strongest correlates were with the amplitude descriptors, standard deviation ($r^2 = 50.1$ %) and maximum amplitude ($r^2 = 41.9$ %). *Halodule* indices were positively correlated with mean salinity, and were negatively correlated with all other parameters. The strongest negative correlation ($r^2 = 40.5$%) was with standard deviation of salinity (a direct measure of fluctuation). Changes in GLI in *Ruppia* correlate best with the frequency descriptors, number of changes per day and significant frequency, although not significant at the $p < 0.001$ level (Table 5-1).

Decreases in shoot number in *Thalassia* correlated strongly with increasing values of amplitude and suddenness of salinity change (Table 5-2). Decreases in *Halodule* shoot number correlated strongly with suddenness of salinity change as well. No statistically significant correlations were observed between changes in *Ruppia* shoot number and the salinity wave descriptors (Table 5-2).

Table 5-1: Pearson product moment correlations between changes in green-leaf index following treatment and salinity wave descriptors. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
Mean Salinity	0.1566	0.3017	-0.0990
	(0.2057)	(0.0131)	(0.4639)
	$r^2 = 2.5\%$	$r^2 = 7.7\%$	$r^2 = 1.0\%$
Standard Deviation of Salinity	**-0.7129**	**-0.6437**	-0.0193
	(0.0000)	**(0.0000)**	(0.8434)
	$r^2 = 50.1\%$	**$r^2 = 40.5\%$**	$r^2 = 0.0\%$
Maximum Amplitude	**-0.6543**	**-0.5810**	0.0268
	(0.0000)	**(0.0000)**	(0.8434)
	$r^2 = 41.9\%$	**$r^2 = 32.7\%$**	$r^2 = 0.0\%$
Suddenness of Salinity Change	**-0.5863**	**-0.5854**	0.1380
	(0.0000)	**(0.0000)**	(0.3061)
	$r^2 = 33.4\%$	**$r^2 = 33.3\%$**	$r^2 = 1.9\%$
Number of Changes Per Day	-0.3056	-0.1262	-0.3195
	(0.0119)	(0.3089)	(0.0154)
	$r^2 = 7.9\%$	$r^2 = 1.6\%$	$r^2 = 8.6\ \%$
Significant Frequency	**-0.3945**	-0.3711	-0.3035
	(0.0010)	(0.0020)	(0.0217)
	$r^2 = 14.3\%$	$r^2 = 12.4\%$	$r^2 = 7.6\%$
Absolute Frequency	**-0.4588**	**-0.4875**	-0.1248
	(0.0001)	**(0.0000)**	(0.3549)
	$r^2 = 19.8\%$	**$r^2 = 22.6\%$**	$r^2 = 1.6\%$

Table 5-2: Pearson product moment correlations between changes in number of shoots following treatment and salinity wave descriptors. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
Mean Salinity	0.0577	0.2508	-0.0218
	(0.6427)	(0.0406)	(0.8721)
	$r^2 = 0.0\%$	$r^2 = 4.8\%$	$r^2 = 0.1\%$
Standard Deviation of Salinity	**-0.5060**	-0.3659	-0.1779
	(0.0000)	(0.0023)	(0.1854)
	$r^2 = 24.5\%$	$r^2 = 12.1\%$	$r^2 = 1.4\%$
Maximum Amplitude	**-0.4469**	**-0.4033**	-0.2559
	(0.0002)	**(0.0007)**	(0.0547)
	$r^2 = 18.7\%$	**$r^2 = 15.0\%$**	$r^2 = 4.9\%$
Suddenness of Salinity Change	**-0.4518**	**-0.4801**	-0.1202
	(0.0001)	**(0.0000)**	(0.3730)
	$r^2 = 19.2\%$	**$r^2 = 21.8\%$**	$r^2 = 0.0\%$
Number of Changes Per Day	-0.0434	0.2322	-0.2363
	(0.7272)	(0.0587)	(0.0768)
	$r^2 = 0.0\%$	$r^2 = 3.9\%$	$r^2 = 3.8\%$
Significant Frequency	-0.3119	0.1571	-0.0503
	(0.0102)	(0.2044)	(0.7104)
	$r^2 = 8.3\%$	$r^2 = 1.0\%$	$r^2 = 0.0\%$
Absolute Frequency	-0.0805	-0.1026	0.1372
	(0.5175)	(0.4087)	(0.3088)
	$r^2 = 0.0\%$	$r^2 = 0.0\%$	$r^2 = 0.9\%$

No statistically significant correlations occurred between rhizome length and the salinity fluctuation descriptors for any seagrass (Table 5-3). *Thalassia* leaf lengths were negatively correlated with all salinity wave descriptors except for mean salinity (Table 5-4). Strongest correlates occurred with absolute frequency and salinity standard deviation. Leaf lengths in *Halodule* were negatively correlated with the amplitude descriptors, standard deviation and maximum amplitude, although not statistically significant. The strongest correlate with *Ruppia* leaf length was the number of salinity changes per day, which correlated negatively, yet not significant at the $p<0.001$ level (Table 5-4).

All salinity fluctuation descriptors negatively correlated with the change in leaf number in *Thalassia* except for mean salinity, the strongest being standard deviation of salinity (Table 5-5). Leaf counts in *Halodule* increased with increasing mean salinity. Changes in leaf number were negatively correlated with the number of salinity changes per day in *Ruppia* (Table 5-5).

Similar to the changes measured by the before to after experiment ratios, the average number of *Thalassia* leaves per shoot after treatment correlated negatively to all salinity descriptors, except for mean salinity (Table 5-6). Once again, the strongest correlate was standard deviation of salinity ($r^2 = 27.5\%$). The average number of leaves per shoot on *Halodule* sprigs increased with increasing mean salinity, but was negatively correlated with increasing standard deviation and number of changes per day. Average leaf counts in *Ruppia* were negatively correlated with the frequency descriptors, number of changes per day and significant frequency (Table 5-6).

Table 5-3: Pearson product moment correlations between changes in rhizome length following treatment and salinity wave descriptors. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
Mean Salinity	0.0191 (0.8781) $r^2 = 0.0\%$	0.1610 (0.1931) $r^2 = 1.1\%$	0.0977 (0.4697) $r^2 = 0.0\%$
Standard Deviation of Salinity	-0.0476 (0.7023) $r^2 = 0.0\%$	0.0660 (0.5912) $r^2 = 0.0\%$	-0.3111 (0.0185) $r^2 = 8.0\%$
Maximum Amplitude	-0.0718 (0.5637) $r^2 = 0.5\%$	-0.1025 (0.4092) $r^2 = 0.0\%$	-0.4044 (0.0018) $r^2 = 14.8\%$
Suddenness of Salinity Change	0.0350 (0.7785) $r^2 = 0.0\%$	-0.0705 (0.5709) $r^2 = 0.0\%$	-0.2534 (0.0572) $r^2 = 4.7\%$
Number of Changes Per Day	-0.3084 (0.0111) $r^2 = 8.1\%$	-0.0375 (0.7631) $r^2 = 0.0\%$	-0.2319 (0.0826) $r^2 = 3.7\%$
Significant Frequency	-0.2099 (0.0883) $r^2 = 3.0\%$	-0.0297 (0.8112) $r^2 = 0.0\%$	-0.0493 (0.7156) $r^2 = 0.0\%$
Absolute Frequency	-0.2934 (0.0160) $r^2 = 7.2\%$	-0.1403 (0.2575) $r^2 = 0.5\%$	0.0959 (0.4781) $r^2 = 0.0\%$

Table 5-4: Pearson product moment correlations between changes in leaf length following treatment and salinity wave descriptors. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
Mean Salinity	0.1660	0.0050	-0.1656
	(0.3497)	(0.9679)	(0.2184)
	$r^2 = 0.0\%$	$r^2 = 0.0\%$	$r^2 = 1.0\%$
Standard Deviation of Salinity	**-0.4740**	-0.3047	-0.0436
	(0.0001)	(0.0122)	(0.7476)
	$r^2 = 21.3\%$	$r^2 = 7.9\%$	$r^2 = 0.0\%$
Maximum Amplitude	**-0.4170**	-0.3226	0.0354
	(0.0004)	(0.0078)	(0.7936)
	$r^2 = 16.1\%$	$r^2 = 9.0\%$	$r^2 = 0.0\%$
Suddenness of Salinity Change	-0.3294	-0.2345	0.1300
	(0.0065)	(0.0562)	(0.3352)
	$r^2 = 9.5\%$	$r^2 = 4.0\%$	$r^2 = 0.0\%$
Number of Changes Per Day	-0.3556	-0.1890	-0.3506
	(0.0031)	(0.1256)	(0.0075)
	$r^2 = 11.3\%$	$r^2 = 2.1\%$	$r^2 = 10.7\%$
Significant Frequency	-0.3233	-0.0738	-0.2407
	(0.0076)	(0.5526)	(0.0713)
	$r^2 = 9.1\%$	$r^2 = 0.0\%$	$r^2 = 4.1\%$
Absolute Frequency	**-0.5063**	0.0002	-0.1020
	(0.0000)	(0.9987)	(0.4504)
	$r^2 = 24.5\%$	$r^2 = 0.0\%$	$r^2 = 0.0\%$

Table 5-5: Pearson product moment correlations between changes in number of leaves per shoot following treatment and salinity wave descriptors. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are in bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
Mean Salinity	0.1904	**0.4160**	-0.2477
	(0.1227)	**(0.0005)**	(0.0632)
	r^2 = 2.1%	r^2 = **16.0%**	r^2 = 4.4%
Standard Deviation of Salinity	**-0.5504**	-0.2559	-0.1893
	(0.0000)	(0.0366)	(0.1585)
	r^2 = **29.2%**	r^2 = 5.1%	r^2 = 1.8%
Maximum Amplitude	**-0.4960**	-0.1740	-0.2761
	(0.0000)	(0.1592)	(0.0376)
	r^2 = **23.4%**	r^2 = 1.5%	r^2 = 5.9%
Suddenness of Salinity Change	**-0.3934**	-0.1408	-0.0625
	(0.0010	(0.2557)	(0.6440)
	r^2 = **14.2%**	r^2 = 0.5%	r^2 = 0.0%
Number of Changes Per Day	-0.3276	-0.1844	**-0.4405**
	(0.0068)	(0.1353)	**(0.0006)**
	r^2 = 9.4%	r^2 = 1.9%	r^2 = **17.9%**
Significant Frequency	-0.3342	-0.1260	-0.1940
	(0.0057)	(0.3096)	(0.1482)
	r^2 = 9.8%	r^2 = 0.1%	r^2 = 2.0%
Absolute Frequency	-0.2481	-0.1199	0.0762
	(0.0429)	(0.3340)	(0.5731)
	r^2 = 4.7%	r^2 = 0.0%	r^2 = 0.0%

Table 5-6: Pearson product moment correlations between number of leaves per shoot following treatment and salinity wave descriptors. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
Mean Salinity	0.1470	0.3672	0.0399
	(0.2352)	(0.0022)	(0.7683)
	$r^2 = 0.7\%$	$r^2 = 12.2\%$	$r^2 = 0.0\%$
Standard Deviation of Salinity	**-0.5345**	-0.2598	-0.0513
	(0.0000)	(0.0338)	(0.7044)
	$r^2 = 27.5\%$	$r^2 = 5.3\%$	$r^2 = 0.0\%$
Maximum Amplitude	**-0.4342**	-0.2105	-0.0245
	(0.0002)	(0.0873)	(0.8567)
	$r^2 = 17.6\%$	$r^2 = 3.0\%$	$r^2 = 0.0\%$
Suddenness of Salinity Change	-0.3337	-0.1253	0.1515
	(0.0058)	(0.3123)	(0.2606)
	$r^2 = 9.8\%$	$r^2 = 0.6\%$	$r^2 = 0.5\%$
Number of Changes Per Day	**-0.4319**	-0.3138	**-0.4937**
	(0.0003)	(0.0097)	**(0.0001)**
	$r^2 = 17.4\%$	$r^2 = 8.5\%$	**$r^2 = 23.0\%$**
Significant Frequency	**-0.4709**	-0.1197	-0.3752
	(0.0001)	(0.3346)	(0.0040)
	$r^2 = 21.0\%$	$r^2 = 0.0\%$	$r^2 = 12.5\%$
Absolute Frequency	**-0.4193**	-0.2253	-0.0436
	(0.0004)	(0.0667)	(0.7472)
	$r^2 = 16.3\%$	$r^2 = 3.6\%$	$r^2 = 0.0\%$

Surprisingly, no statistically significant correlations were found between the salinity descriptors and *Thalassia* aboveground and belowground biomass or *Halodule* whole sprig biomass (Table 5-7). The only statistically significant correlate occurred with *Ruppia* sprig biomass and the number of changes in salinity per day (Table 5-7).

Correlations with Temperature, Light and Water Clarity

The percent light reaching seagrass depth was the strongest correlation with green-leaf indices of seagrasses in the stable salinity treatments, although not statistically significant (Table 5-8). This correlation was negative for both *Thalassia* and *Ruppia* plants, positive for *Halodule.* The number of *Thalassia* shoots increased with increasing light intensities, however (Table 5-9). Shoot number in *Halodule* increased with higher fractions of light reaching the seagrass depth. None of the physical parameters correlated with changes in rhizome length for *Thalassia* or *Halodule*, however the percent light reaching seagrass depth positively correlated with *Ruppia* rhizome length with a p-value of 0.056 and r^2 of 17% (Table 5-9).

Leaf lengths of *Halodule* in the stable salinity treatment correlated with increasing intensities of light at seagrass depth. Leaf lengths in *Ruppia* correlated negatively with increasing water clarity. *Thalassia* leaf lengths did not correlate with any of the temperature or light measurements (Table 5-10).

The changes in leaf length did not correlate with statistical significance for any of the variables for any seagrass (Table 5-10, top panel). Mean temperature and light intensity were strong correlates with increases in *Ruppia* leaves per shoot and average number of leaves after treatment (Table 5-10, middle and bottom panels).

Table 5-7: Pearson product moment correlations between biomass measurements following treatment and salinity wave descriptors. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are bold ($p<0.001$).

	Thalassia (shoots)	*Thalassia* (rhizome)	*Halodule*	*Ruppia*
Mean Salinity	-0.0270	-0.0551	-0.0470	-0.0420
	(0.8281)	(0.6579)	(0.7059)	(0.7564)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 0.0%	r^2 = 0.0%
Standard Deviation of Salinity	0.0959	-0.1082	-0.2295	0.0334
	(0.4402)	(0.3834)	(0.0617)	(0.8050)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 3.8%	r^2 = 0.0%
Maximum Amplitude	0.0099	-0.1876	-0.3176	-0.0679
	(0.9365)	(0.1285)	(0.0088)	(0.6159)
	r^2 = 0.0%	r^2 = 2.0%	r^2 = 8.7%	r^2 = 0.0%
Suddenness of Salinity Change	0.0991	-0.1464	-0.2634	0.1739
	(0.4250)	(0.2373)	(0.0312)	(0.1957)
	r^2 = 0.0%	r^2 = 0.6%	r^2 = 5.5%	r^2 = 1.3%
Number of Changes Per Day	-0.2467	-0.0688	-0.0393	**-0.4888**
	(0.0441)	(0.5802)	(0.7520)	**(0.0001)**
	r^2 = 4.6%	r^2 = 0.0%	r^2 = 0.0%	**r^2 = 22.5%**
Significant Frequency	0.0789	0.1247	0.1268	-0.1242
	(0.5259)	(0.3148)	(0.3065)	(0.3572)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 0.1%	r^2 = 0.0%
Absolute Frequency	-0.1990	0.0636	0.1482	0.0461
	(0.1065)	(0.6091)	(0.2314)	(0.7336)
	r^2 = 2.5%	r^2 = 0.0%	r^2 = 070%	r^2 = 0.0%

Table 5-8: Stable salinity treatment Pearson product moment correlations between changes in green-leaf index, temperature and light. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
Mean temperature	-0.2292	0.1839	0.2001
	(0.3451)	(0.4512)	(0.4414)
	$r^2 = 0.0\%$	$r^2 = 0.0\%$	$r^2 = 0.0\%$
Intensity of Light at Seagrass Depth	-0.1632	0.1170	-0.0162
	(0.5043)	(0.6334)	(0.9506)
	$r^2 = 0.0\%$	$r^2 = 0.0\%$	$r^2 = 0.0\%$
Percent Light Reaching Seagrass Depth	-0.3854	0.4336	-0.5420
	(0.1032)	(0.0636)	(0.0246)
	$r^2 = 9.8\%$	$r^2 = 14.0\%$	$r^2 = 24.7\%$

Table 5-9: Stable salinity treatment Pearson product moment correlations between changes in number of shoots, rhizome length, temperature and light. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are bold ($p<0.001$).

Number of Shoots

	Thalassia	*Halodule*	*Ruppia*
Mean temperature	0.3978	-0.1121	0.4214
	(0.0917)	(0.6477)	(0.0920)
	r^2 = 10.9%	r^2 = 0.0%	r^2 = 12.3%
Intensity of Light at	0.5618	-0.0980	0.2482
Seagrass Depth	(0.0123)	(0.6899)	(0.3367)
	r^2 = 27.5%	r^2 = 0.0%	r^2 = 0.0%
Percent Light Reaching	0.2995	0.5769	0.2206
Seagrass Depth	(0.2128)	(0.0097)	(0.3948)
	r^2 = 3.6%	r^2 = 29.4%	r^2 = 0.0%

Rhizome Length

	Thalassia	*Halodule*	*Ruppia*
Mean temperature	0.1914	0.0685	0.2170
	(0.4325)	(0.7805)	(0.4028)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 0.0%
Intensity of Light at	0.0695	-0.0531	0.3347
Seagrass Depth	(0.7774)	(0.8291)	(0.1891)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 5.3%
Percent Light Reaching	0.1188	0.2202	0.4719
Seagrass Depth	(0.6281)	(0.3650)	(0.0558)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 17.1%

Table 5-10: Stable salinity treatment Pearson product moment correlations between changes in leaf length, number of leaves and average number of leaves, temperature and light measurements.

Leaf Length

	Thalassia	*Halodule*	*Ruppia*
Mean temperature	0.2650	0.3723	0.3317
	(0.2728)	(0.1165)	(0.1933)
	r^2 = 1.6%	r^2 = 8.8%	r^2 =5.1%
Intensity of Light at Seagrass Depth	0.0404	0.5369	0.2219
	(0.8695)	(0.0178)	(0.3920)
	r^2 = 0.0%	r^2 = 24.6%	r^2 = 0.0%
Percent Light Reaching Seagrass Depth	-0.2890	-0.0589	-0.6423
	(0.2302)	(0.8108)	(0.0054)
	r^2 = 3.0%	r^2 = 0.0%	r^2 = 37.3%

Number of Leaves per Shoot

	Thalassia	*Halodule*	*Ruppia*
Mean temperature	0.1004	0.4985	**0.7723**
	(0.6826)	(0.0298)	**(0.0003)**
	r^2 = 0.0%	r^2 = 20.4%	**r^2 = 60.0%**
Intensity of Light at Seagrass Depth	0.2212	0.3771	**0.7846**
	(0.3627)	(0.1115)	**(0.0002)**
	r^2 = 0.0%	r^2 = 9.2%	**r^2 = 59.0%**
Percent Light Reaching Seagrass Depth	-0.1054	(0.0676)	0.3162
	(0.6675)	(0.7833)	(0.2163)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 4.0%

Average Number of Leaves per Shoot

	Thalassia	*Halodule*	*Ruppia*
Mean temperature	0.6257	0.4130	**0.7937**
	(0.0042)	(0.0789)	**(0.0001)**
	r^2 = 35.6%	r^2 = 12.2%	**r^2 = 60.5%**
Intensity of Light at Seagrass Depth	0.5137	0.2729	0.6058
	(0.0245)	(0.2583)	(0.0100)
	r^2 = 22.1%	r^2 =2.0%	r^2 = 32.5%
Percent Light Reaching Seagrass Depth	-0.2325	-0.0567	-0.0396
	(0.3381)	(0.8177)	(0.8802)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 0.0%

No statistically significant correlates occurred between biomass and temperature and light, although *Thalassia* aboveground biomass correlated strongest with increasing temperature (Table 5-11). In addition to being influenced by temperature, belowground *Thalassia* biomass correlated with both light intensities and fractions at the seagrass depth. *Halodule* and *Ruppia* whole sprig biomasses were influenced by light as well, correlating positively with light percent and intensity, respectively (Table 5-11).

Correlations with Water Nutrient Concentrations

Changes in green-leaf indices of *Thalassia* in the stable salinity treatment correlated with total phosphorus ($r^2 = 39.7\%$), orthophosphate (52.5%) and total dissolved phosphorus (51.0%) concentrations (Table 5-12), although not statistically significant at the $p < 0.001$ level. *Halodule* green-leaf indices did not correlate with any of the nutrient concentration measured. A positive relationship existed between *Ruppia* green-leaf indices and total Kjeldahl nitrogen (TKN) concentrations (Table 5-12).

No statistically significant correlates occurred between changes in seagrass shoot number and nutrient concentrations. The strongest correlation in *Thalassia* was with concentration of total dissolved phosphorus (Table 5-13). Shoot numbers in *Halodule* correlated negatively with TKN and nitrite concentrations, however a positive correlation existed with nitrate. A negative correlation between TKN and *Ruppia* was the strongest relationship found between changes in rhizome length and nutrient concentrations, although not statistically significant (Table 5-14).

Table 5-11: Stable salinity treatment Pearson product moment correlations between biomass measurements, temperature and light. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are bold ($p<0.001$).

	Thalassia (shoots)	*Thalassia* (rhizome)	*Halodule*	*Ruppia*
Mean temperature	0.4228	0.4000	0.0754	0.4256
	(0.0713)	(0.0897)	(0.7590)	(0.0885)
	r^2 = 13.0%	r^2 = 16.0%	r^2 = 0.0%	r^2 = 12.7%
Intensity of Light at Seagrass Depth	0.2655	0.4809	0.2062	0.5418
	(0.2720)	(0.0371)	(0.3970)	(0.0247)
	r^2 = 1.6%	r^2 = 18.6%	r^2 = 0.0%	r^2 = 24.7%
Percent Light Reaching Seagrass Depth	0.3399	0.5074	0.4669	0.1136
	(0.1545)	(0.0266)	(0.0439)	(0.6641)
	r^2 = 6.4%	r^2 = 21.4%	r^2 = 17.2%	r^2 = 0.0%

Table 5-12: Stable salinity treatment Pearson product moment correlations between changes in green-leaf index and nutrient concentrations. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
TP	0.6656	-0.4142	0.1220
	(0.0094)	(0.1410)	(0.6777)
	r^2 = 39.7%	r^2 = 10.2%	r^2 = 0.0%
PO4	0.6871	-0.3806	0.2514
	(0.0195)	(0.1794)	(0.3860)
	r^2 = 52.5%	r^2 = 7.4%	r^2 = 0.0%
TDP	0.7400	-0.4075	-0.0115
	(0.0025)	(0.1481)	(0.9688)
	r^2 = 51.0%	r^2 = 9.7%	r^2 = 0.0%
TKN	0.0368	-0.0409	**0.7820**
	(0.9006)	(0.8897)	**(0.0010)**
	r^2 = 0.0%	r^2 = 0.0%	**r^2 = 57.9%**
NH4	0.3823	-0.3290	-0.3916
	(0.2459)	(0.3232)	(0.2336)
	r^2 = 5.1%	r^2 = 0.9%	r^2 = 5.9%
NO3+2	-0.2910	-0.1787	-0.0415
	(0.3128)	(0.5991)	(0.9036)
	r^2 = 0.8%	r^2 = 0.0%	r^2 = 6.2%
NO2	-0.0011	0.1441	0.4656
	(0.9970)	(0.6231)	(0.0934)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 15.2%
DIN	-0.3091	0.0356	-0.5422
	(0.2823)	(0.9038)	(0.0452)
	r^2 = 2.0%	r^2 = 0.0%	r^2 = 23.5%

Table 5-13: Stable salinity treatment Pearson product moment correlations between changes in number of shoots following and nutrient concentrations. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
TP	0.4183	-0.2492	-0.4364
	(0.1367)	(0.3903)	(0.1187)
	r^2 = 10.6%	r^2 = 0.0%	r^2 = 12.3%
PO4	0.5064	-0.4776	-0.2908
	(0.0646)	(0.0842)	(0.3132)
	r^2 = 19.4%	r^2 = 16.4%	r^2 = 0.8%
TDP	0.6077	-0.1900	-0.3591
	(0.0212)	(0.5152)	(0.2073)
	r^2 = 31.7%	r^2 = 0.0%	r^2 = 5.6%
TKN	-0.4271	-0.7391	-0.0739
	(0.1277)	(0.0025)	(0.8017)
	r^2 = 11.4%	r^2 = 50.8%	r^2 = 0.0%
NH4	0.2909	0.5454	-0.4549
	(0.3856)	(0.0827)	(0.1597)
	r^2 = 0.0%	r^2 = 21.9%	r^2 = 11.9%
NO3+2	-0.2144	0.5977	-0.2384
	(0.5266)	(0.0240)	(0.4117)
	r^2 = 0.0%	r^2 = 30.4%	r^2 = 0.0%
NO2	-0.0931	-0.5935	0.3342
	(0.7515)	(0.0253)	(0.2429)
	r^2 = 0.0%	r^2 = 29.8%	r^2 = 3.8%
DIN	-0.0650	0.4363	-0.4435
	(0.8494)	(0.1797)	(0.1719)
	r^2 = 0.0%	r^2 = 10.0%	r^2 = 10.7%

Table 5-14: Stable salinity treatment Pearson product moment correlations between changes in rhizome length and nutrient concentrations. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
TP	-0.2779	-0.3416	0.2045
	(0.3360)	(0.2320)	(0.4832)
	r^2 = 0.0%	r^2 = 4.3%	r^2 = 0.0%
PO4	-0.1531	-0.3244	0.1710
	(0.6013)	(0.2577)	(0.5589)
	r^2 = 0.0%	r^2 = 3.1%	r^2 = 0.0%
TDP	-0.2792	-0.3478	0.3787
	(0.3337)	(0.2231)	(0.1818)
	r^2 = 0.1%	r^2 = 4.8%	r^2 = 7.2%
TKN	0.2045	-0.0126	-0.6662
	(0.4830)	(0.9659)	(0.0093)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 39.8%
NH4	-0.3980	-0.2455	0.3741
	(0.2254)	(0.4669)	(0.2570)
	r^2 = 6.4%	r^2 = 0.0%	r^2 = 4.4%
NO3+2	-0.2590	0.0190	0.0970
	(0.4418)	(0.9485)	(0.7416)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 0.0%
NO2	0.3553	0.1076	-0.3367
	(0.2125)	(0.7142)	(0.2392)
	r^2 = 5.3%	r^2 = 0.0%	r^2 = 3.9%
DIN	-0.3138	-0.1571	0.0672
	(0.3474)	(0.6445)	(0.8445)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 0.0%

Halodule leaf lengths had strong positive correlations with orthophosphate and total dissolved phosphorus, with r^2 values of 75.7 and 82.3%, respectively (Table 5-15). *Ruppia* leaf lengths had a statistically significant, positive correlation with total Kjeldahl nitrogen concentration. A strong, yet statistically insignificant, negative correlation occurred with ammonium concentration and *Ruppia* leaf length (Table 5-15).

Although no statistically significant correlates occurred between changes in leaf number and nutrient concentrations, the changes in leaf number in *Thalassia* were influenced by concentrations of TP, PO4, and TDP, with r^2 values 36.9, 47.4, and 51.0%, respectively (Table 5-16). Average leaf numbers per shoot after treatments were negatively correlated nitrate concentrations for all seagrasses (Table 5-17). Strong, positive correlations occurred between *Halodule* and *Ruppia* leaf number and nitrite concentrations, although similar negative relationships occurred with ammonium and dissolved inorganic nitrogen concentrations for both species (Table 5-17).

Correlations with Field Conditions at Collection Sites

Initial green-leaf indices were higher in *Thalassia* when conditions in Little Madeira Bay were more saline and experienced less salinity standard deviation (Table 5-18). Green-leaf indices in *Halodule* decreased with increasing salinity and increased with higher salinity standard deviations. No correlations were seen with *Ruppia* starting green-leaf index.

Higher salinities also correlated strongly (r^2 = 76.9 and 71.1%)with more *Thalassia* leaves per shoot (Table 5-19) and longer leaf lengths, although not statistically significant at the $p < 0.001$ level (Table 5-20). Higher salinities correlated with more

leaves per shoot in *Ruppia* (Table 5-19), where increased salinity standard deviations led to taller leaves (Table 5-20). No statistically significant relationships were seen involving *Halodule* leaf numbers or lengths.

Table 5-15: Stable salinity treatment Pearson product moment correlations between changes in leaf length and nutrient concentrations. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are in bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
TP	-0.2221	0.7730	0.2920
	(0.4453)	(0.0012)	(0.3111)
	r^2 = 0.0%	r^2 =56.4%	r^2 = 0.9%
PO4	0.0230	**0.8806**	0.5422
	(0.9378)	**(0.0000)**	(0.0452)
	r^2 = 0.0%	**r^2 = 75.7%**	r^2 = 23.5%
TDP	-0.2542	**0.9145**	0.2343
	(0.3805)	**(0.0000)**	(0.4200)
	r^2 = 0.0%	**r^2 = 82.3%**	r^2 = 0.0%
TKN	0.5767	-0.1381	**0.7874**
	(0.0308)	(0.6377)	**(0.0008)**
	r^2 = 27.7%	r^2 = 0.0%	**r^2 = 58.8%**
NH4	-0.6663	0.4799	-0.7491
	(0.0252)	(0.1352)	(0.0080)
	r^2 = 38.2%	r^2 = 14.5%	r^2 = 51.2%
NO3+2	-0.5441	-0.3451	-0.6560
	(0.0443)	(0.2269)	(0.0108)
	r^2 = 23.7%	r^2 = 4.6%	r^2 =38.3%
NO2	0.6802	-0.0450	0.6380
	(0.0074)	(0.8785)	(0.0141)
	r^2 = 41.8%	r^2 = 0.0%	r^2 = 35.8%
DIN	-0.5203	0.0669	-0.6225
	(0.1008)	(0.8456)	(0.0408)
	r^2 = 19.0%	r^2 = 0.0%	r^2 = 32.0%

Table 5-16: Stable salinity treatment Pearson product moment correlations between changes in number of leaves per shoot and nutrient concentrations. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are in bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
TP	0.6458	-0.1557	0.0211
	(0.0126)	(0.5950)	(0.9429)
	r^2 = 36.9%	r^2 = 0.0%	r^2 = 0.0%
PO4	0.7170	0.1203	0.3446
	(0.0039)	(0.6820)	(0.2275)
	r^2 = 47.4%	r^2 = 0.0%	r^2 = 4.5%
TDP	0.7401	-0.0632	0.2554
	(0.0025)	(0.8302)	(0.3782)
	r^2 = 51.0%	r^2 = 0.0%	r^2 = 0.0%
TKN	-0.0657	0.1967	-0.1889
	(0.8234)	(0.5002)	(0.5177)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 0.0%
NH4	0.4057	-0.3734	-0.3550
	(0.2157)	(0.0640)	(0.2841)
	r^2 = 7.2%	r^2 = 25.7%	r^2 = 2.9%
NO3+2	-0.2520	-0.5889	-0.6867
	(0.3847)	(0.0267)	(0.0067)
	r^2 = 0.0%	r^2 = 29.2%	r^2 = 42.8%
NO2	-0.0534	0.5878	0.4957
	(0.8562)	(0.0271)	(0.0715)
	r^2 = 0.0%	r^2 = 29.1%	r^2 = 18.3%
DIN	0.1017	-0.6334	-0.6491
	(0.7662)	(0.0364)	(0.0307)
	r^2 = 0.0%	r^2 = 33.5%	r^2 = 35.7%

Table 5-17: Stable salinity treatment Pearson product moment correlations between the number of leaves per shoot and nutrient concentrations. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are in bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
TP	0.2655	-0.1268	-0.2363
	(0.3590)	(0.6659)	(0.4161)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 0.0%
PO4	0.6215	0.2767	0.1756
	(0.0177)	(0.3383)	(0.5481)
	r^2 = 33.5%	r^2 = 0.0%	r^2 = 0.0%
TDP	0.3379	-0.0302	-0.1397
	(0.2374)	(0.9184)	(0.6339)
	r^2 = 4.0%	r^2 = 0.0%	r^2 = 0.0%
TKN	0.5224	0.4505	0.4452
	(0.0553)	(0.1060)	(0.1107)
	r^2 = 21.2%	r^2 = 13.7%	r^2 = 13.1%
NH4	-0.6592	**-0.8473**	**-0.9101**
	(0.0274)	**(0.0010)**	**(0.0004)**
	r^2 = 37.2%	**r^2 = 68.7%**	**r^2 = 80.1%**
NO3+2	**-0.8577**	**-0.8909**	**-0.8911**
	(0.0001)	(0.0000)	(0.0000)
	r^2 = 71.4%	**r^2 = 77.7%**	**r^2 = 77.7%**
NO2	0.7383	**0.8908**	**0.9300**
	(0.0026)	(0.0000)	(0.0000)
	r^2 = 50.7%	**r^2 = 76.7%**	**r^2 = 85.4%**
DIN	-0.8205	**-0.8970**	**-0.9288**
	(0.0020)	(0.0002)	(0.0000)
	r^2 = 63.7%	**r^2 = 78.3%**	**r^2 = 84.7%**

Table 5-18: Pearson product moment correlations between initial green-leaf indices and mean salinity and temperature during the month prior to collection, as well as the salinity at collection. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are in bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
Mean Salinity	**0.8158**	**-0.7009**	0.1198
	(0.0000)	**(0.0008)**	(0.6469)
	r^2 = 64.6%	**r^2 = 46.1%**	r^2 = 0.0%
Standard Deviation of Salinity	-0.5386	0.5338	0.3650
	(0.0174)	(0.0186)	(0.1497)
	r^2 = 24.8%	r^2 = 24.3%	r^2 = 7.5%
Mean Temperature	0.1637	-0.0399	0.4634
	(0.5030)	(0.8712)	(0.0610)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 16.2%
Salinity Measured at Collection	**0.7260**	**-0.8197**	-0.3645
	(0.0004)	**(0.0000)**	(0.1503)
	r^2 = 52.7%	**r^2 = 65.3%**	r^2 = 1.5%

Table 5-19: Pearson product moment correlations between initial number of leaves per shoot and mean salinity and temperature during the month prior to collection, as well as the salinity at collection. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are in bold ($p<0.001$).

	Thalassia	*Halodule*	*Ruppia*
Mean Salinity	0.9028	0.6552	0.8914
	(0.0137)	(0.1578)	(0.0423)
	$r^2 = 76.9\%$	$r^2 = 28.7\%$	$r^2 = 72.6\%$
Standard Deviation of Salinity	-0.1759	0.4652	-0.6460
	(0.7389)	(0.3525)	(0.2389)
	$r^2 = 0.0\%$	$r^2 = 2.1\%$	$r^2 = 22.3\%$
Mean Temperature	0.1876	0.4228	-0.1168
	(0.7219)	(0.4036)	(0.8516)
	$r^2 = 0.0\%$	$r^2 = 0.0\%$	$r^2 = 0.0\%$
Salinity Measured at Collection	0.5022	0.1847	0.8818
	(0.3101)	(0.7261)	(0.0479)
	$r^2 = 6.5\%$	$r^2 = 0.0\%$	$r^2 = 70.3\%$

Table 5-20: Pearson product moment correlations between original leaf lengths and mean salinity and temperature during the month prior to collection, as well as the salinity at collection. P- values are given in parentheses. R-squared values are given as well. Statistically significant correlates are in bold ($p < 0.001$).

	Thalassia	*Halodule*	*Ruppia*
Mean Salinity	0.8770	0.5516	0.1071
	(0.0218)	(0.2565)	(0.8639)
	r^2 = 71.1%	r^2 = 13.0%	r^2 = 0.0%
Standard Deviation of Salinity	0.1781	0.4468	0.8785
	(0.7357)	(0.3743)	(0.0499)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 69.6%
Mean Temperature	0.4543	0.4477	0.7290
	(0.3654)	(0.3734)	(0.1623)
	r^2 = 0.8%	r^2 = 0.1%	r^2 = 37.5%
Salinity Measured at Collection	0.3187	-0.1789	-0.0546
	(0.5381)	(0.7346)	(0.9305)
	r^2 = 0.0%	r^2 = 0.0%	r^2 = 0.0%

CHAPTER 6
PRODUCTIVITY AND LEAF OSMOLALITY- EXPERIMENT 8

Introduction

Salinity fluctuation is detrimental to the existence and production of green leaves necessary for photosynthesis, as seen in the previous seven experiments. How primary productivity is affected in the surviving leaves is unknown. Seagrass leaves damaged by fluctuating salinities may have lower rates of productivity due to reductions in photosynthetically viable cells.

Many studies have looked at the effect of decreases in salinity on productivity in seagrasses (Barbour 1970, Hammer 1968, and Hellblom and Bjork 1999); however, none address the effects of salinity fluctuation. Lower salinities appear to inhibit photosynthetic activity in seagrasses, not because of reduced salt concentrations, but due to reduced inorganic carbon content. If seawater is diluted with water rich in bicarbonates, assimilation rates are higher (Hammer 1968).

Seagrasses exposed to salinity fluctuation treatments should exhibit impaired productivity. By exposing seagrasses to various degrees of light intensity and measuring oxygen evolution, an oxygen evolution vs. light curve can be created in order to assess affinity of light and primary productivity. From what was seen in the facility experiments, it is expected that *Thalassia* and *Halodule* will have reduced rates of oxygen evolution in salinity fluctuation treatments with high amplitude and frequency. In

addition, *Ruppia* should have impaired productivity in the high frequency salinity fluctuation treatments.

The ability to survive unfavorable salinities depends heavily on the osmotic adjustment within the tissues of the plant (Flowers et al. 1986). Since seagrasses lack salt-secreting glands, they osmoregulate through their epidermal cells (Jagels 1973, 1983). These epidermal cells have highly invaginated plasmalemmas (semipermeable layers of cell protoplasm) with numerous mitochondria situated within, and are closely analogous to the basal cells of salt glands in *Spartina* and osmoregulatory cells of marine invertebrates (Jagels 1973). Ions are actively transported into channels associated with the plasmalemmas, using an ATP-requiring transport mechanism. In a study of the temperate seagrass *Zostera marina,* protons (H^+ ions) were found to be the major driving ions for this transport mechanism (Fernandez et al. 1999). Other studies of *Ruppia* (Brock 1981, Durako 2000), found that amino acid accumulation may function as a salt tolerance mechanism. The accumulation of amino acids, such as proline, raises the osmotic potential of the cytoplasm to counterbalance the cell vacuolar solute levels caused by changes in external salinities (Brock 1981). The ability for a seagrass to osmoregulate, as well as the energy requirements necessary for ion transport, may be critical for a seagrass to survive fluctuating salinities, especially if salinity change is frequent.

The resilience of *Ruppia* in the facility experiments leads one to expect a high degree of osmoregulation when exposed to fluctuating salinities. Impaired osmoregulation is expected in *Thalassia* and *Halodule*, especially in treatments with high amplitudes and frequencies of salinity fluctuation.

Materials and Methods

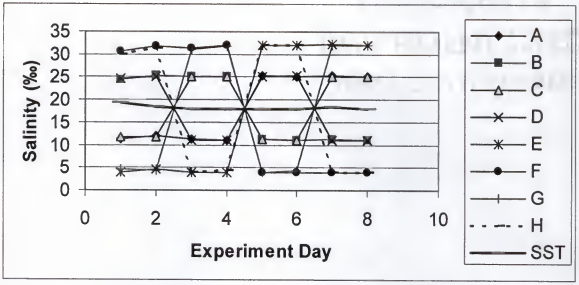
Seagrasses were collected from the same site as the six previous experiments. The plants were transported in aerated coolers to Gainesville, Florida where they were acclimated in an approximately 1.1 m^3 round polyethylene tub initially filled with water of the same salinity as the water in which they were collected (14‰). Purified seawater was diluted with aged tap water to achieve a salinity of 14‰. The seawater (of approximately 35‰) used in Gainesville was collected from the Atlantic Ocean at Marineland, Florida, and subsequently treated in a recirculating seawater purification system consisting of a sand filter, UV sterilizer, and biofilter. Salinity in the tub was gradually adjusted to 18‰ by the addition of seawater over a 63 day period.

Twenty-seven 13 liter white polyethylene buckets (without lids) were used as experimental tanks. Nine treatments were applied that differed in wave amplitude (4 to 32 ‰ and 11 to 25 ‰ salinity ranges), frequency (1/4 and 1/8 day), final salinity (low or high) and a stable 18 ‰ salinity (Figure 6-1, bottom panel). Three replicate buckets were used per treatment. Within each bucket, two sprigs each of *Thalassia*, *Halodule,* and *Ruppia* were placed- one for oxygen evolution vs. light determination, the other for osmolality measurements. The seagrass sprigs were not planted but were left suspended in the water. All buckets were covered by a 70% shadecloth. *Thalassia* and *Halodule* sprigs were selected by the same criteria used in the facility experiments. Selected *Ruppia* sprigs had at least 15 shoots and a growing rhizome tip.

The experimental treatments were applied for 9 days, beginning on May 7, 2000. Treatments were divided into two groups and staggered by one day due to the time necessary for data collection. The two groups were 1) high frequency treatments (Codes

C, D, G, and H) and 2) low frequency and stable salinity treatments (Codes A, B, E, F, and SST). A visual survey was not done during the course of the treatments, however a green leaf index was assessed prior to and following the treatments. After the treatment period, seagrasses from the same bucket were randomly selected into groups for oxygen evolution/uptake vs. light and osmolarity measurements.

In order to create an oxygen evolution/uptake vs. light curve, selected seagrass sprigs were placed into 300 ml BOD bottles in water of 18‰ salinity. The BOD bottles were placed in a water bath to keep temperature constant. Treatments for oxygen evolution vs. light included in the absence of light (bottles wrapped in foil) and various light intensities (10%, 30%, and 100% of full sun) using shade cloths placed over the entire water bath. Dissolved oxygen concentrations were measured (YSI 57 DO Meter with a BOD Electrode) initially and approximately every hour until a stable measurement was achieved. Each plant was exposed to every light level for approximately an hour, starting from dark and increasing light incrementally. Following a dissolved oxygen measurement, a layer of shadecloth was gradually folded back over the water bath to expose the seagrasses to increased light intensities. Once all samples in a bath were measured, the shadecloth layer was removed. Blank BOD bottles were measured in all light levels as well so productivity in the incubation water could be estimated and subtracted. Sunlight was measured using a quantum photometer (Li-Cor Inc. model LI-190). Fractions of sunlight received in the treatments were estimated by dividing the mean sunlight during the treatment period by the amount of shading provided by the shadecloths.



Treatment	Amplitude (‰)	Period (Days)	Mean (‰)	Final Exposure
A	7	8	18	High Salinity (25‰)
B	7	8	18	Low Salinity (11‰)
C	7	4	18	High Salinity (25‰)
D	7	4	18	Low Salinity (11‰)
E	14	8	18	High Salinity (32‰)
F	14	8	18	Low Salinity (4‰)
G	14	4	18	High Salinity (32‰)
H	14	4	18	Low Salinity (4‰)
SST	0	0	18	No Step (18‰)

Figure 6-1: Salinity patterns (top panel) and treatment codes (bottom panel) for Experiment 8.

During the first phase of this experiment, the automatic stirrers attached to the BOD electrode ceased to spin. In order to maintain proper circulation of water, the BOD bottles were agitated manually by vigorous shaking. This method was used for the remainder of the experiment.

Internal leaf osmolality was determined using a vapor pressure osmometer (Wescor Vapro 5100). Sprigs were taken from their respective treatments and placed in water of 18 ‰ salinity. Following at least three but up to six hours at this salinity, the leaves were blotted dry and cut in 6 mm sections to fill the chamber of the osmometer. In most cases, one section of *Thalassia*, and three sections of *Halodule* and *Ruppia* were used to fill the chamber. Osmolality was then measured. Three replicates (a section from a new seagrass leaf from a replicate bucket) were used for each treatment.

Data Analysis for Experiment 8

Oxygen evolution/uptake vs. light curves were created by charting dissolved oxygen measurements normalized by leaf surface area with estimated light intensities received during the period. On the first day, measurements were taken on the low amplitude-high frequency and high amplitude-high frequency treatments. On the second day, measurements were made on the low amplitude-low frequency, high amplitude-low frequency, and stable salinity treatments.

Various aspects of the oxygen evolution/uptake vs. light curves were analyzed, including oxygen uptake in the dark, maximum net oxygen evolution, net oxygen evolution at the light intensity of 400 $\mu E*m^{-2}*sec^{-1}$ (a similar light intensity experienced by the seagrasses on both days), gross oxygen evolution (calculated from the dark oxygen

uptake and the maximum net oxygen evolution), and the alpha, or affinity to low light (estimated as the slope of the oxygen evolution/uptake vs. light curve between no light and the lowest light level). One-way ANOVAs (Fisher's LSD procedure) were run to compare the responses by each species to each treatment with regard to the aforementioned aspects of the oxygen evolution vs. light curves. The significance threshold was set at $p < 0.05$. One-way ANOVAs were also used to identify differences among means of osmolalities.

Results

Salinity patterns for the nine treatments in Experiment 8 are given in Figure 6-1. Mean temperature for all treatments was approximately 28°C.

Oxygen Evolution/Uptake vs. Light Intensity

Oxygen evolution/uptake vs. light curves for *Thalassia* are given in Figure 6-2. Each line represents a replicate for each treatment. Samples measured on the second day (Treatments A, B, E, F, and SST) received a greater intensity of light, with full sun intensities of approximately 975$\mu E*m^{-2}*sec^{-1}$, whereas those on the first day were approximately 407 $\mu E*m^{-2}*sec^{-1}$.

Oxygen uptake was greatest in *Thalassia* in the high amplitude/low frequency treatment ending with high salinity (Treatment E); oxygen uptake rates in the other treatments did not statistically differ from those measured in the stable salinity treatments (Figure 6-3, top panel). Maximum net oxygen evolution and oxygen evolution measured at 400 $\mu E*m^{-2}*sec^{-1}$ did not statistically differ amongst treatments (Figure 6-3, second and third panels).

Thalassia gross oxygen evolution was greatest in Treatment E as well (Figure 6-4, top panel). Gross oxygen evolution in this treatment was not statistically greater than the other treatments, except for the low amplitude, low frequency Treatment A. Affinity at low light levels was lowest in the low amplitude, low frequency treatments (Treatments A and B) and the stable salinity treatment (Figure 6-4, middle panel).

Oxygen evolution/uptake vs. light curves for *Halodule* are given in Figure 6-5. Greatest dark oxygen uptakes occurred in the high amplitude, low frequency treatments, Treatments E and F (Figure 6-6, top panel). In general, greatest maximum net oxygen evolutions in *Halodule* occurred in the high amplitude, high frequency treatments (Treatments G and H) and Treatment F, the high amplitude, low frequency, low final salinity treatment (Figure 6-6, middle panel). Oxygen evolution at 400 $\mu E*m^{-2}*sec^{-1}$ was greatest in treatments with high frequency and final exposure to low salinities, Treatments D and H (Figure 6-6, third panel).

Halodule gross oxygen evolution was statistically greater in the high amplitude, low frequency, low final salinity treatment (Treatment F) than that measured in other treatments (Figure 6-7, top panel). Affinity for low light was least in the stable salinity and low amplitude, low frequency treatments, A and B (Figure 6-7, middle panel).

Oxygen evolution/uptake vs. light curves for *Ruppia* are given in Figure 6-8. Dark oxygen uptake was greatest in *Ruppia* in the high amplitude, low frequency, low ending salinity Treatment F (Figure 6-9, top panel). Greatest *Ruppia* net oxygen evolutions occurred in the low amplitude, low frequency, high ending salinity treatment (Treatment A) and the stable salinity treatment (Figure 6-9, second panel). Net oxygen evolutions at 400 $\mu E*m^{-2}*sec^{-1}$ were similar amongst treatments, except for Treatment B

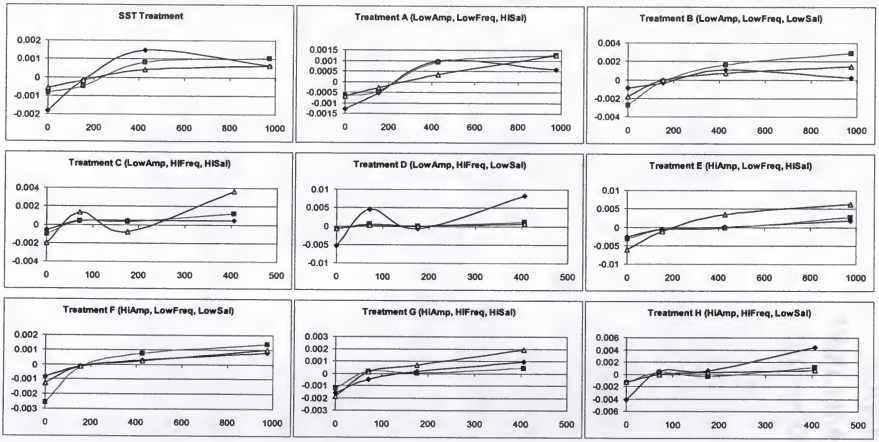
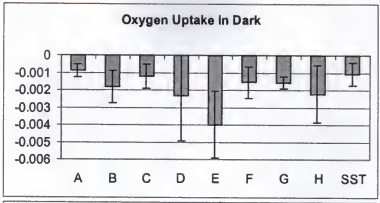
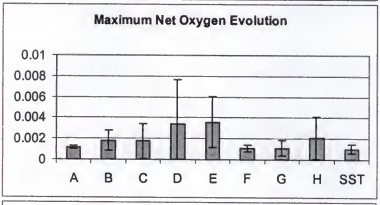
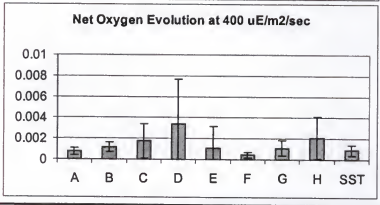


Figure 6-2: Oxygen Evolution/Uptake vs. Light Curves for *Thalassia*. X-axis units are light intensity ($\mu E/m^2/sec$), Y-axis units are dissolved oxygen (mg O_2/l*min*cm^2 leaf surface area). Treatments are described by magnitude of amplitude and frequency, as well as final salinity exposure prior to 18‰.

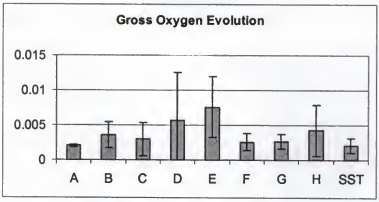


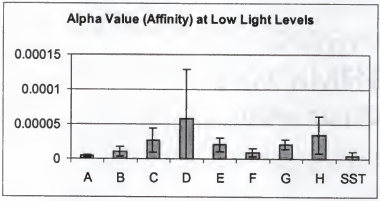




Treatment	Amp.	Freq.	Final Sal.	Dark Uptake ANOVA Gp.	Max Evolution ANOVA Gp.	Evol. At 400 ANOVA Gp.
A	7 ‰	1/8 d	25 ‰	B	A	A
B	7 ‰	1/8 d	11 ‰	AB	A	A
C	7 ‰	1/4 d	25 ‰	B	A	A
D	7 ‰	1/4 d	11 ‰	AB	A	A
E	14 ‰	1/8 d	32 ‰	A	A	A
F	14 ‰	1/8 d	4 ‰	B	A	A
G	14 ‰	1/4 d	32 ‰	B	A	A
H	14 ‰	1/4 d	4 ‰	AB	A	A
SST	0 ‰	0 d	18 ‰	B	A	A

Figure 6-3: *Thalassia* dark oxygen uptake, maximum oxygen evolution, and net oxygen evolution for treatments in Experiment 8. Y axis units are dissolved oxygen (mg O_2/l*min*cm^2 leaf surface area). Error bars are standard deviations. Overlaps of letters in ANOVA groups signify no statistical difference at 95% confidence level.





Treatment	Amp.	Freq.	Final Sal.	Gross Evol. ANOVA Gp.	Affinity ANOVA Gp.
A	7 ‰	1/8 d	25 ‰	A	A
B	7 ‰	1/8 d	11 ‰	AB	A
C	7 ‰	1/4 d	25 ‰	AB	AB
D	7 ‰	1/4 d	11 ‰	AB	B
E	14 ‰	1/8 d	32 ‰	B	AB
F	14 ‰	1/8 d	4 ‰	AB	A
G	14 ‰	1/4 d	32 ‰	AB	AB
H	14 ‰	1/4 d	4 ‰	AB	AB
SST	0 ‰	0 d	18 ‰	AB	A

Figure 6-4: *Thalassia* gross oxygen evolution and affinity at low light levels for treatments in Experiment 8. Y axis units for gross oxygen evolution are dissolved oxygen (mg O_2/l*min*cm^2 leaf surface area). Y-axis units for affinity are dissolved oxygen per light intensity (mg O_2/l*min*cm^2 leaf surface area / $\mu E*m^{-2}*sec^{-1}$). Error bars are standard deviations. Overlaps of letters in ANOVA groups signify no statistical difference at 95% confidence level.

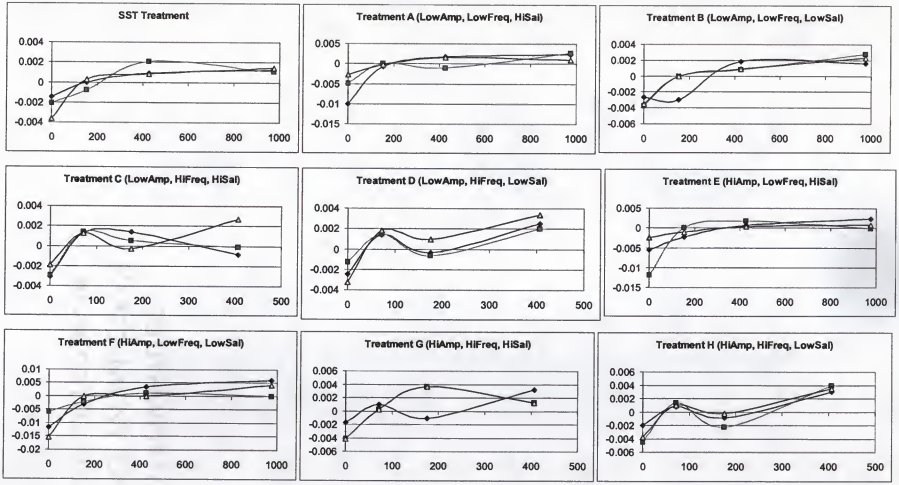
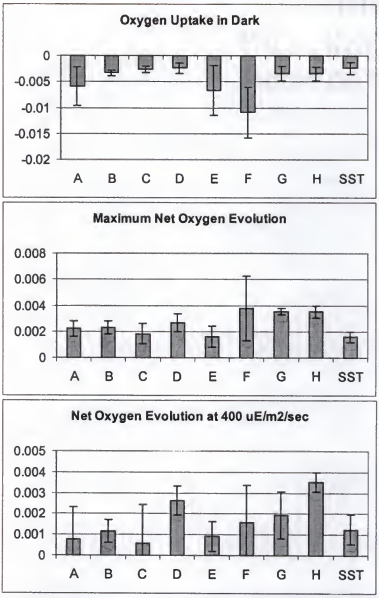
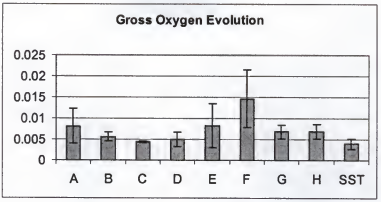


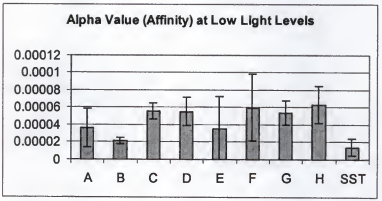
Figure 6-5: Oxygen Evolution/Uptake vs. Light Curves for *Halodule*. X-axis units are light intensity ($\mu E/m^2/sec$), Y-axis units are dissolved oxygen (mg O_2/l*min*cm^2 leaf surface area). Treatments are described by magnitude of amplitude and frequency, as well as final salinity exposure prior to 18‰.



Treatment	Amp.	Freq.	Final Sal.	Dark Uptake ANOVA Gp.	Max Evolution ANOVA Gp.	Evol. At 400 ANOVA Gp.
A	7 ‰	1/8 d	25 ‰	B	ABC	AB
B	7 ‰	1/8 d	11 ‰	B	ABC	AB
C	7 ‰	1/4 d	25 ‰	B	AB	A
D	7 ‰	1/4 d	11 ‰	B	ABC	BC
E	14 ‰	1/8 d	32 ‰	AB	A	AB
F	14 ‰	1/8 d	4 ‰	A	C	ABC
G	14 ‰	1/4 d	32 ‰	B	BC	ABC
H	14 ‰	1/4 d	4 ‰	B	BC	C
SST	0 ‰	0 d	18 ‰	B	A	AB

Figure 6-6: *Halodule* dark oxygen uptake, maximum oxygen evolution, and net oxygen evolution for treatments in Experiment 8. Y-axis units are dissolved oxygen (mg O_2/l*min*cm^2 leaf surface area). Error bars are standard deviations. Overlaps of letters in ANOVA groups signify no statistical difference at 95% confidence level.





Treatment	Amp.	Freq.	Final Sal.	Gross Evol. ANOVA Gp.	Affinity ANOVA Gp.
A	7 ‰	1/8 d	25 ‰	A	ABC
B	7 ‰	1/8 d	11 ‰	A	AB
C	7 ‰	1/4 d	25 ‰	A	BC
D	7 ‰	1/4 d	11 ‰	A	BC
E	14 ‰	1/8 d	32 ‰	A	ABC
F	14 ‰	1/8 d	4 ‰	B	C
G	14 ‰	1/4 d	32 ‰	A	BC
H	14 ‰	1/4 d	4 ‰	A	C
SST	0 ‰	0 d	18 ‰	A	A

Figure 6-7: *Halodule* gross oxygen evolution and affinity at low light levels for treatments in Experiment 8. Y-axis units for gross oxygen evolution are dissolved oxygen (mg O_2/l*min*cm^2 leaf surface area). Y-axis units for affinity are dissolved oxygen per light intensity (mg O_2/l*min*cm^2 leaf surface area / $\mu E*m^{-2}*sec^{-1}$). Error bars are standard deviations. Overlaps of letters in ANOVA groups signify no statistical difference at 95% confidence level.

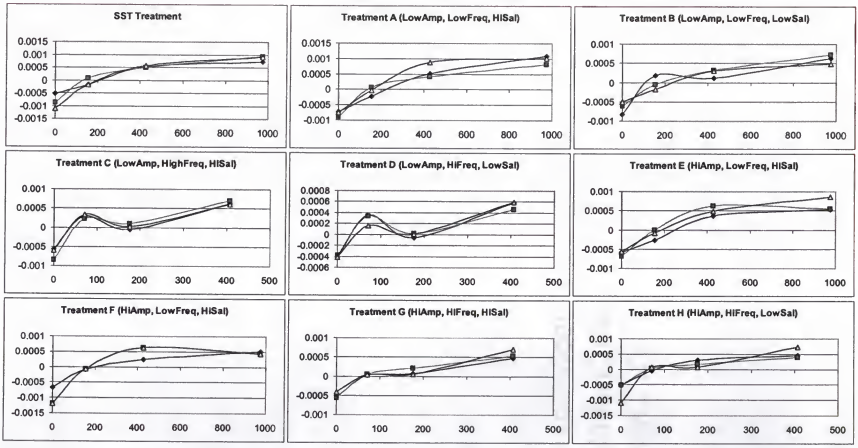
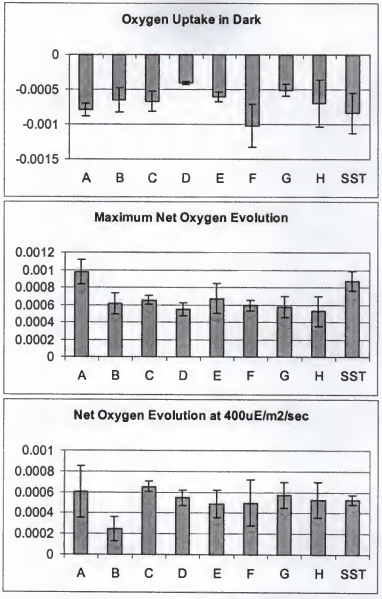
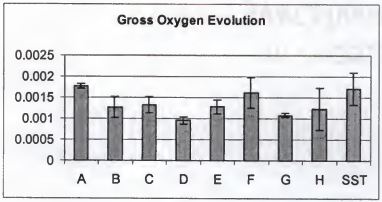


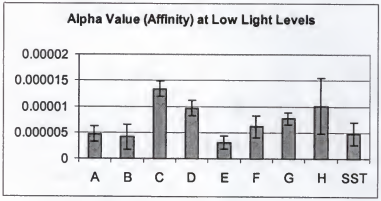
Figure 6-8: Oxygen Evolution/Uptake vs. Light Curves for *Ruppia*. X-axis units are light intensity ($\mu E/m^2/sec$), Y-axis units are dissolved oxygen (mg O_2/l*min*cm^2 leaf surface area). Treatments are described by magnitude of amplitude and frequency, as well as final salinity exposure prior to 18‰.



Treatment	Amp.	Freq.	Final Sal.	Dark Uptake ANOVA Gp.	Max Evolution ANOVA Gp.	Evol. At 400 ANOVA Gp.
A	7 ‰	1/8 d	25 ‰	AB	C	B
B	7 ‰	1/8 d	11 ‰	BC	A	A
C	7 ‰	1/4 d	25 ‰	BC	A	B
D	7 ‰	1/4 d	11 ‰	C	A	B
E	14 ‰	1/8 d	32 ‰	BC	AB	AB
F	14 ‰	1/8 d	4 ‰	A	A	B
G	14 ‰	1/4 d	32 ‰	BC	A	B
H	14 ‰	1/4 d	4 ‰	ABC	A	B
SST	0 ‰	0 d	18 ‰	AB	BC	B

Figure 6-9: *Ruppia* dark oxygen uptake, maximum oxygen evolution, and net oxygen evolution for treatments in Experiment 8. Y-axis units are dissolved oxygen (mg O_2/l*min*cm^2 leaf surface area). Error bars are standard deviations. Overlaps of letters in ANOVA groups signify no statistical difference at 95% confidence level.





Treatment	Amp.	Freq.	Final Sal.	Gross Evol. ANOVA Gp.	Affinity ANOVA Gp.
A	7 ‰	1/8 d	25 ‰	D	AB
B	7 ‰	1/8 d	11 ‰	ABC	AB
C	7 ‰	1/4 d	25 ‰	ABCD	D
D	7 ‰	1/4 d	11 ‰	A	CD
E	14 ‰	1/8 d	32 ‰	ABC	A
F	14 ‰	1/8 d	4 ‰	BCD	ABC
G	14 ‰	1/4 d	32 ‰	A	BC
H	14 ‰	1/4 d	4 ‰	AB	CD
SST	0 ‰	0 d	18 ‰	CD	AB

Figure 6-10: *Ruppia* gross oxygen evolution and affinity at low light levels for treatments in Experiment 8. Y-axis units for gross oxygen evolution are dissolved oxygen (mg O_2/l*min*cm^2 leaf surface area). Y-axis units for affinity are dissolved oxygen per light intensity (mg O_2/l*min*cm^2 leaf surface area / $\mu E*m^{-2}*sec^{-1}$). Error bars are standard deviations. Overlaps of letters in ANOVA groups signify no statistical difference at 95% confidence level.

which was significantly lower (Figure 6-9, third panel). Affinity to low light in *Ruppia* was greatest in the treatments with a high frequency of salinity fluctuation (Treatment C,D, G, and H) (Figure 6-10, middle panel). All other treatments had similar affinities.

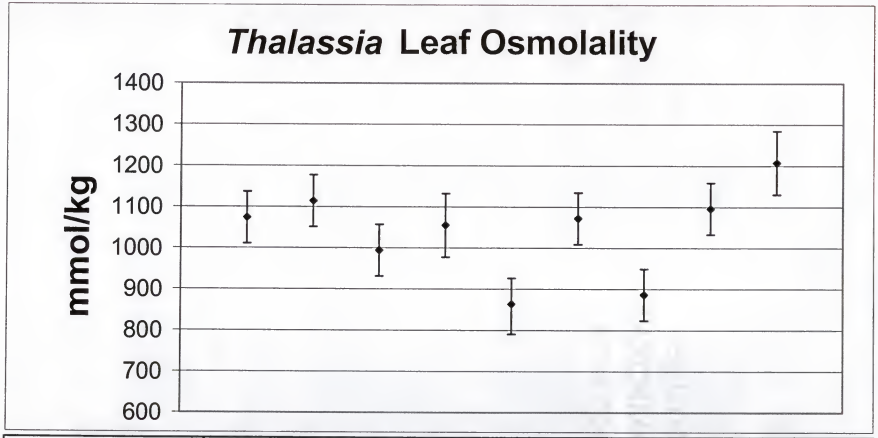
Internal Leaf Osmolality

Highest osmotic concentrations were measured in *Thalassia* sprigs from the stable salinity treatment (Figure 6-6). For each combination of amplitude and frequency, seagrasses with recent exposure to lower salinities had greater leaf osmolalities than those with recent exposure to higher salinities. Internal leaf osmolalities of those exposed to the high amplitude treatment that had recent exposure to high salinities (Treatments E and G) were significantly lower than those with recent exposure to low salinities (F and H).

Halodule osmolalities were highest among treatments exposed to low salinities for each amplitude-frequency combination (Figure 6-7). In high frequency treatments, sprigs with recent exposure to low salinities had significantly higher internal leaf osmolalities than those exposed to higher salinities (Treatments C vs. D and G vs. H). No significant differences in *Ruppia* osmolality occurred amongst treatments (Figure 6-8), although those in the stable salinity treatment had the greatest mean osmolality.

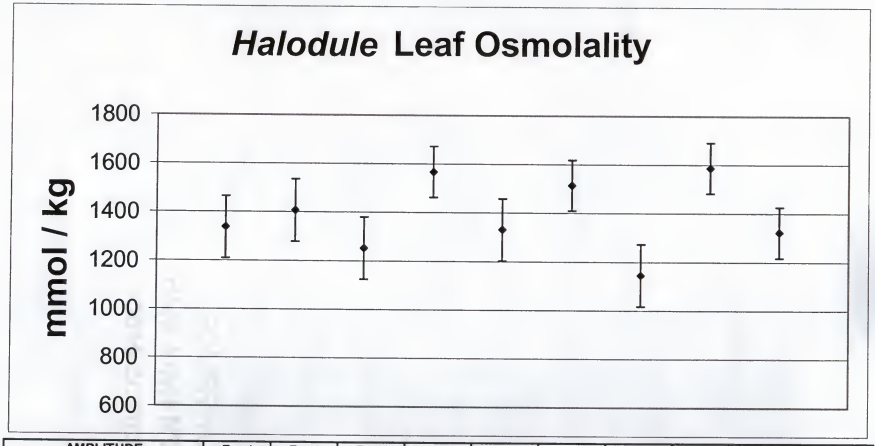
Discussion

The increase in oxygen evolution measured in seagrasses exposed to salinity fluctuation treatments could be due in part to two mechanisms. The loss of outside leaves on a seagrass shoot led to increased rates of primary production in the remaining



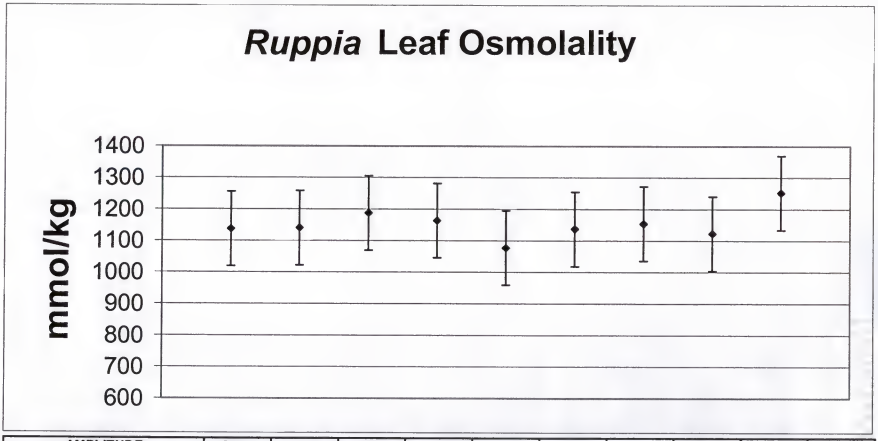
AMPLITUDE	7 ppt	7 ppt	7 ppt	7 ppt	14 ppt	14 ppt	14 ppt	14 ppt	0 ppt
FREQUENCY	1/8 day	1/8 day	1/4 day	1/4 day	1/8 day	1/8 day	1/4 day	1/4 day	0 day
FINAL TREATMENT SAL	25 ppt	11 ppt	25 ppt	11 ppt	32 ppt	4 ppt	32 ppt	4 ppt	18 ppt
TREATMENT CODE	A	B	C	D	E	F	G	H	SST

Figure 6-6: Internal leaf osmolality of *Thalassia* in treatments of Experiment 8. Osmotic concentrations of water of salinity of 18‰ ranged between 512 and 524 mmol/kg.



AMPLITUDE	7 ppt	7 ppt	7 ppt	7 ppt	14 ppt	14 ppt	14 ppt	14 ppt	0 ppt
FREQUENCY	1/8 day	1/8 day	1/4 day	1/4 day	1/8 day	1/8 day	1/4 day	1/4 day	0 day
FINAL TREATMENT SAL	25 ppt	11 ppt	25 ppt	11 ppt	32 ppt	4 ppt	32 ppt	4 ppt	18 ppt
TREATMENT CODE	A	B	C	D	E	F	G	H	SST

Figure 6-7: Internal leaf osmolality of *Halodule* in treatments of Experiment 8. Osmotic concentrations of water of salinity of 18‰ ranged between 512 and 524 mmol/kg.



AMPLITUDE	7 ppt	7 ppt	7 ppt	7 ppt	14 ppt	14 ppt	14 ppt	14 ppt	0 ppt
FREQUENCY	1/8 day	1/8 day	1/4 day	1/4 day	1/8 day	1/8 day	1/4 day	1/4 day	0 day
FINAL TREATMENT SAL	25 ppt	11 ppt	25 ppt	11 ppt	32 ppt	4 ppt	32 ppt	4 ppt	18 ppt
TREATMENT CODE	A	B	C	D	E	F	G	H	SST

Figure 6-8: Internal leaf osmolality of *Ruppia* in treatments of Experiment 8. Osmotic concentrations of water of salinity of 18‰ ranged between 512 and 524 mmol/kg.

undamaged leaves in a study by Tomasko and Dawes (1989). Remaining leaves, even if undamaged by salinity fluctuation, could be compensating for those lost in the salinity fluctuation treatment. Higher affinities to light in seagrasses exposed to stressful treatments (such as *Ruppia* in the high frequency treatments) may be due to this compensatory mechanism.

Secondly, salinity fluctuation may in fact have damaged these leaves, and impaired their internal lacunal system, which would allow normally stored oxygen to escape, giving the impression of greater primary production. Normally, as production increases during the day, *Thalassia* leaves swell to as much as 200-250% their morning volumes, due to the internal production of gases at a much greater rate than can be exchanged through the leaf surface (Zieman 1974). If seagrasses cannot store metabolic gases adequately, oxygen will be released to the water column during light, increasing measured dissolved oxygen concentrations.

Osmolality in *Ruppia* leaf tissues changed within one minute of exposure to a new salinity (Durako 2000). Since osmolalities in Experiment 8 were measured between three to six hours following exposure to a new salinity, the initial change in *Ruppia* was probably missed. The exact time of exposure to the new salinity was not recorded for seagrasses in this experiment and should be documented in further osmolality studies.

All seagrasses tested appear to osmoregulate rather than osmoconform, since internal leaf osmolalities were twice that of the ambient water for all seagrasses exposed to the stable salinity treatment. Osmolalities measured in this study were assumed to be that of the internal extracellular fluids. Seagrasses must adjust the ionic concentrations of their internal fluids when ambient salinity changes. The rate of osmoregulation is also

important. The rapid acclimation rate of osmoregulation in *Ruppia* may account for its resiliency in the salinity fluctuation experiments. Osmoregulation appears to be much slower in *Thalassia* and *Halodule* than in *Ruppia.* Slower acclimation rates may be costly to seagrass survival, especially when salinity is constantly changing. When salinity fluctuates often, as in the facility experiments or within the northern land margin of Florida Bay, the costs may exceed the gains, causing leaf mortality. With repeated occurrence, fewer and fewer resources will be available for production of new plant structure or propagules, affecting the distribution and abundances of these species.

In general, the oxygen evolution/uptake vs. light curves and the osmoregulatory capabilities of the seagrasses reaffirm their distributions in the estuary. *Thalassia,* with the highest affinity to light and the lowest ability to osmoregulate, prefers deeper waters with more stable salinities. *Halodule,* with intermediate affinity, is found in more shallow waters than *Thalassia*. *Ruppia,* with a lower affinity to light than *Halodule* and *Thalassia* and the highest degree of osmoregulation, is commonly found in shallower waters with variable salinity. Furthermore, *Ruppia* oxygen evolution/uptake vs. light curves rarely approached saturation, indicating a preference for high light levels. *Ruppia* also had the lowest oxygen evolution per unit leaf area. *Ruppia* has a low investment in rhizomous storage, allocating more resources to leaf and seed production, which may be energetically less expensive.

CHAPTER 7
DISCUSSION

Overall Discussion

Salinity fluctuation has a decisively negative effect on seagrass survival and community development. Fluctuations in salinity may be more important than mean salt concentration in determining the distribution of euryhaline macrophytes in estuarine waters (Montague and Ley 1993). The frequent phenomenon of salinity change may cause the sparse seagrass communities within the ponds and bays of the northern land margin of Florida Bay and elsewhere in seagrass dominated estuaries.

Seagrass survival may be impaired by the metabolic costs incurred by osmoregulation in response to salinity fluctuation. When submerged macrophytes are exposed to a change in salinity, turgor is maintained by pumping ions through potassium and chloride channels (Fernandez et al. 1999). If the new salinity is higher, ions must be pumped inward to maintain osmotic balance; if the new salinity is lower, ions are pumped outward. In addition, amino acids are used to osmoregulate. The use of organic molecules to achieve osmotic balance diverts production away from growth and reproduction. Active processes, such as pumping ions and amino acid formation, have energetic costs.

The loss of photosynthetic tissue as a result of salinity fluctuation further exacerbates the metabolic toll of frequent osmoregulation. Seagrass rhizomes serve as

storage sites for carbohydrates and proteins used for leaf regeneration (Valentine et al. 1997). If these storages are frequently tapped in order to make new leaves to replace damaged ones without adequate replenishment, the plant could die.

Distributions and abundances of seagrasses are, in turn, influenced by depletion of rhizomatous resources. Sexual reproduction is infrequent in *Thalassia* and *Halodule* (McMillan and Mosely 1967). Reproduction is these species is primarily achieved through vegetative growth. For this to occur, viable plants must be present. Starch reserves in rhizomes must not only allow regeneration of leaves, but are necessary for rhizome extension.

Ruppia, on the other hand, has very thin rhizomes. High fecundity and rapid growth increase its chances of survival (Kantrud 1991), and create its ephemeral boom and bust cycles of abundance. In addition, *Ruppia* has an advanced seasonal growth cycle compared to *Thalassia* and *Halodule,* initiating growth in the winter, prior to the other seagrasses (Lazar and Dawes 1991).

Transplantation, like salinity fluctuation, can lead to physiological shock in seagrasses (Bird et al. 1993, Van Tussenbroek 1996). The general defoliation and chlorosis experienced by seagrasses during the first 10 days in all experiments (including stable salinity) may be a result of handling during transplantation. Transplantation may also have made the test sprigs more susceptible to salinity fluctuation, but the recovery in some treatments (especially in stable salinity) suggests that the stress associated with transplantation would be overcome. The initial leaf loss and chlorosis of all plants in most treatments need not affect the interpretation of the affect of salinity fluctuations

more than would be caused by, perhaps, one or two additional cycles of salinity fluctuation.

The range, amplitude, and frequency of salinity fluctuation are major determinants of seagrass survival. The better survival of *Thalassia* and *Halodule* in more marine salinities is well documented anecdotally (Phillips 1960, Zieman 1975), and to a lesser degree, experimentally (McMahan 1968). The better survival of these species in fluctuation regimes at higher average salinities is further experimental evidence of marine salinity as a more appropriate environmental condition for these two species.

Salinity is non-optimal for seagrass survival and growth more often when the amplitude of fluctuation increases. The loss of photosynthetic material and plant structure in these plants was not as severe when the amplitude of fluctuation waves was smaller, possibly in part because the ambient salinities were closer to their optimal ranges.

As shown in simulations of the hypothetical effects of salinity on seagrasses (Fears 1993, Anastasiou 1999), longer lag times in acclimation dampen the amplitude of internal osmolality fluctuations, but also increase the time the plants internal osmolality differs from that in its surrounding water. The resulting greater osmotic potential may directly damage cells through cell bursting or shrinkage.

Ruppia has the widest salinity range of any angiosperm (Brock 1981, Kantrud 1991). Despite its tolerance to wide ranges of salinity, the frequency of salinity changes were detrimental to its survival. Defoliation, chlorosis, and higher affinities to light associated with exposures to high frequency salinity fluctuations may provide insight into the osmoregulatory mechanisms of this species. In spite of *Ruppia's* ability to

osmoregulate rapidly and possibly to compensate for stress by using more light, it may still be energetically costly if the need for osmoregulation is frequent. *Ruppia* has little rhizomous storage to affect this process.

The susceptibility of *Ruppia* to salinity fluctuation with high frequency in the facility experiments was contrary to what was found in the pilot study. When the salinity regime of the pilot study was repeated in Experiment 5, *Ruppia* survival was impaired in the high frequency treatment (p4d), as indicated by decreases in green-leaf index and the morphometric measurements. The seagrasses in the pilot study were subjected to higher temperatures and more light than in Experiment 5. In addition, the pilot study was conducted during July, near the beginning of *Ruppia's* die back cycle (Lazar and Dawes 1991). The mechanism responsible for *Ruppia's* respondes in the pilot study is not clear, however.

Light and nutrient availability are of secondary importance in habitats of extreme salinity fluctuation, especially for species sensitive to salinity fluctuation such as *Thalassia*. Seagrasses require a high intensity of light for photosynthesis (Zieman and Wetzel 1980). Biweekly light measurements in Little Madeira Bay ranged between 100 and 1600 $\mu E / m^2$ sec, with a mean intensity of 647 $\mu E / m^2$ sec (Chesnes 1999). *Thalassia* was resilient, however, when exposed to severe, periodic light limitations (Kraemer and Hanisak 2000). The survival of *Thalassia* and *Halodule* is better under reduced light, but stable salinity treatments versus those in full sun, and a low amplitude salinity fluctuation regime is experimental evidence of the secondary importance of light in environments exposed to salinity fluctuation.

Seagrasses in northern Florida Bay are limited by phosphorus (Fourqurean and Zieman 1992, Montague and Ley 1993). Phosphorus concentrations in the test facility were an order of magnitude higher than that from the collection site, Little Madeira Bay, during 1996: 14.9μg / l (Rudnick et al. 1999). Average total nitrogen concentrations into Little Madeira Bay during 1996 were 1180 μg / l (Rudnick et al. 1999), slightly higher than the water used in the test facility. Despite the elevated phosphorus concentrations, the seagrasses in the test facility succumbed to the affects of salinity fluctuation. Whether low nutrient concentrations exacerbate the affects of salinity fluctuation is unknown, but plausible due to the high metabolic demands associated with withstanding highly variable salinity regimes.

An odor of sulfide was evident in water pumped from the seawater supply well. Although sulfide concentrations were not measured, sulfide toxicity was probably not a factor in the facility experiments. Seagrasses in the tropics have been thought to be susceptible to sulfide toxicity, since the biogenic carbonate sediments have low iron content, so the production of iron-sulfide compounds, such as pyrite, is relatively limited compared to the iron-rich sediments of much of the U.S. east coast (Erskine and Koch 2000). Short-term (48 hour) exposures to root level sulfide concentrations (ranging from 2.0 to 10 mM) failed to produce any visual signs of sulfide toxicity in *Thalassia testudinum*, although leaf elongation decreased with increased sulfide concentrations (Erskine and Koch 2000). These concentrations are well above the odor threshold for humans (Camp 1968). *Halodule wrightii* has a wide tolerance to sediment sulfides in comparison to *Ruppia maritima* (Erskine and Koch 2000). Given the success of *Ruppia* in the facility experiments, it is assumed that sulfide toxicity was not a factor. The better

growth of *Halodule* and *Ruppia* in the bubbled tanks is probably due to better circulation of water around the leaves and higher light rather than reductions in sulfide. Further, the sediments used in the facility experiments were coarser and probably less anaerobic than in the field, therefore sulfide toxicity in the field would seem more likely than in the facility.

Conclusions

As hypothesized by Montague and Ley (1993), and confirmed by this study, in habitats where salinity fluctuation is common, as in the ponds and bays of the northern land margin of Florida Bay, salinity fluctuation may be the single most important factor dictating the distribution and abundance of submerged macrophytes. Even in cases where nutrient, light, and temperature levels are optimal, seagrasses will be impaired if salinity fluctuates frequently over a wide range.

Fluctuations in salinity of sufficient magnitude occur often at the northern land margin of Florida Bay. In estuaries, salinity fluctuations are often associated with the onset of the wet season, tropical storms, drought, or, as seen in the shallow basins of Florida Bay, a simple change in wind direction. The timing and attenuation of water releases is an important consideration in water management for a variety of reasons including BOD, nutrient loading, and fish migration. Salinity fluctuation must also be added to the list. Holding back water in times of drought as well as excessive releases in times of water abundance will exacerbate the spatial range of salinity fluctuation, affecting areas (upstream or downstream from areas of normal salinity fluctuation) whose organisms may not have the ability to adapt.

The position of maximum salinity fluctuation in the estuary is dynamic, especially under various quantities of water discharge. In the northwest fork of the Loxahatchee estuary, the saltwater-freshwater interface can shift approximately 3 to 5 river miles as a result of changes in freshwater inflow, compared to a 0.5 to 1.5 mile shift due to tidal or seasonal influences (Russell and McPherson 1984).

> For any estuary there is a rate of freshwater flow that will push the band of favorable salinities beyond estuarine boundaries into open waters, eliminating favorable habitat entirely. Likewise, for every estuary there is a freshwater flow so low that the band of favorable salinities retreats upriver where the area of favorable habitat is small. (Browder and Moore 1981, p. 421)

For mobile organisms, zones of unfavorable salinity can be dealt with by simply moving to a more favorable area. Immobile organisms must either adapt or die. For immobile organisms such as *Thalassia* and *Halodule* that rely on vegetative growth as the main method of reproduction, the latter option will diminish distributions significantly.

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BIOGRAPHICAL SKETCH

Thomas Chesnes, the youngest of six children was born on June 20, 1973, in Palm Beach Gardens, Florida, to Rosemarie and Gerard Chesnes. Growing up in south Florida, he acquired a deep appreciation for the outdoors, especially marine environments.

Thomas graduated from Palm Beach Gardens High School in June 1991, and later pursued an undergraduate degree at the University of Florida. Finally deciding on the major of zoology, he received his bachelor's degree with high honors in 1995. Here he had his first exposure to research, studying osmoregulation in estuarine fish for his senior thesis, under the guidance of Dr. Frank Nordlie.

Thomas received his master's degree from the Department of Environmental Engineering Sciences in 1999. His research focused on the responses of fouling organisms to habitats of varying salinity in the northern land margin of Florida Bay. He continued his research in this system, studying macrophytes in an experimental facility for his doctoral dissertation. After graduation, he will serve as an Assistant Professor of Biology at Palm Beach Atlantic College in West Palm Beach, Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Clay L. Montague, Chairman
Associate Professor of Environmental Engineering Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Thomas L. Crisman
Professor of Environmental Engineering Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

J. Stephen Davis
Professor of Botany

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Frank G. Nordlie
Professor Emeritus of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Evan Chipouras
Associate Professor of Biology
University of Tampa

This dissertation was submitted to the Graduate Faculty of the College of Engineering and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 2002

Pramod P. Khargonekar
Dean, College of Engineering

Winfred M. Phillips
Dean, Graduate School